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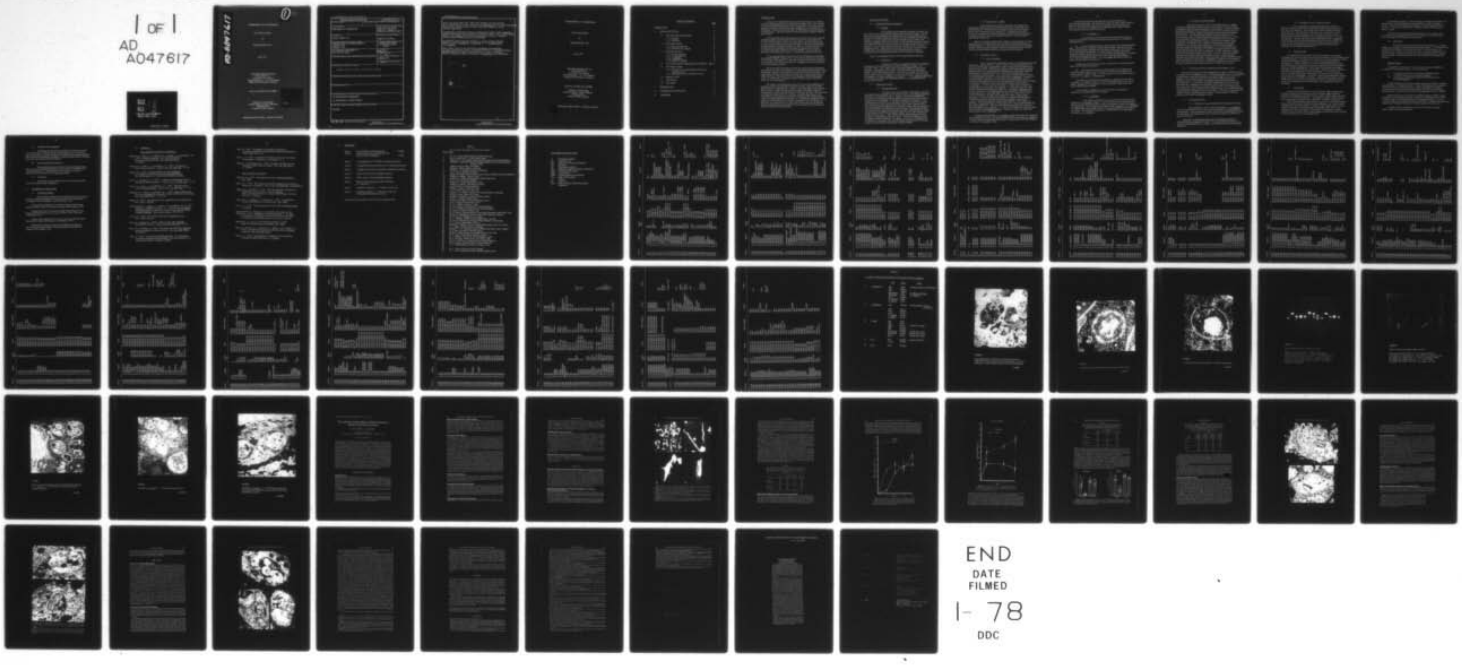

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CHEMOTHERAPY OF LEISHMANIASIS

Final Technical Report

By

WALLACE PETERS, M.D.

March 1977

EUROPEAN RESEARCH OFFICE
United States Army
London NW1 5TH, England
(Sponsoring Agency: U.S. Army Medical
Research and Development Command)

Grant No. DA-ERO-124-74-G0049

Department of Parasitology
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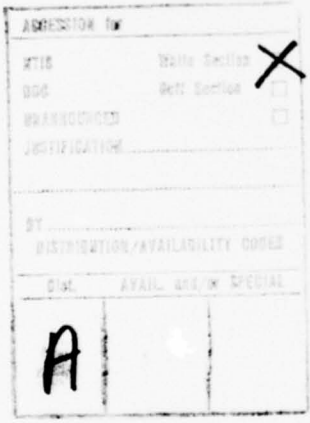
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

During the period March 1976 - March 1977 progress has been reported in (1) cultivation of a number of species of Leishmania, and (2) strain maintenance utilizing the cryobank which now holds nearly 600 isolates at liquid nitrogen temperature.

A comparative ultrastructual study of Noguchi-Adler bodies showed interesting external differences and intracellulor structural changes. Also, ultrastructural studies of L. hertigi have revealed paracrystalline arrays of ribosome-like particles, situated close to the karyosome.

Considerable progress has been reported in the biochemical taxonomic studies of African Leishmania; "L. donovani s.l.", L. braziliensis, and L. hertigi.

Other studies report on the interaction Leishmania and macrophages in virto; immunological studies, including, the effect of immunopotentiating and immunosuppressive drugs, and immunological status of laboratory workers; metabolic studies of amastigotes of Leishmania; and chemotherapy.



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INTRODUCTION

During his visit to Walter Reed Army Institute of Research in July 1976 the Principal Investigator handed over a comprehensive progress review of all activities in the field of leishmaniasis research at the Liverpool School. This report included studies on biochemical taxonomy of the genus Leishmania, cultivation and strain maintenance, ultrastructure and immunology, as well as the host-parasite interaction and chemotherapy. The last two topics were those specifically supported under the present Grant.

In the belief that this overall picture of our activities is of value to the staff of WRAIR and other interested parties of the US Army we are submitting for this, our Final Technical Report, a report in a similar format. The work carried out by Dr. Lewis on Grant support has now been terminated, and the results are presented in several publications (starred in Section 3.2, page 9). A copy of the proof of the latest and most comprehensive of these papers is enclosed (the manuscript having been approved for publication earlier by WRAIR), as well as a copy of a preliminary note indicating work which it is hoped will be followed up this year.

A new grant application concentrating on the chemotherapy of leishmaniasis was submitted to WRAIR in February 1977. At the time of writing news is awaited on the outcome of this application. Meanwhile research continues as far as possible with support from other sources, although this is inadequate to allow continuation of our full chemotherapy programme.

We are fortunate to have been joined by Mrs. Paula Stenning (supported by the Wellcome Trust) who will contribute to our chemotherapy programme several years' postgraduate experience at the Rockefeller University on cellular immunology. This is particularly important at this stage of our studies on the mode of action of anti-leishmanial drugs. Other members of the team engaged on chemotherapy research are Dr. Carol Homewood, Mr. Reginaldo Brazil (of Rio de Janeiro), and Chief Technician, Mr. Brian Robinson.

The scope of our chemotherapy research will in future be broadened by new associations that we have been able to develop with other scientists in Liverpool. Weekly discussions organised by Mr. R. Sells, a consultant surgeon at the Liverpool Royal Infirmary, on aspects of cell-mediated immunity with special reference to leishmaniasis are clearly a valuable aid in our thinking on this topic. We have started a collaborative project with Dr. A. Griffiths, a consultant dermatologist at the Royla Infirmary, on aspects of the non-specific activation of the immune system as a means of combating leishmanial infections. Dr. Griffiths has considerable clinical experience of leishmaniasis in Iran and has maintained his links with that country. Recently a joint project with Dr. R. New of the Liverpool University Biochemical Department has been started on introducing antimony compounds into liposomes as a means of rendering them lysosomotropic.

1. SCIENTIFIC ACTIVITIES

1.1 Cultivation and strain maintenance

1.1.1 Cryobank

The cryobank has been expanded considerably during the last year so that we now have nearly 600 isolates preserved in liquid nitrogen. The main source of the material added this year has been the transfer of important reference material from the WHO Leishmaniasis Reference Centre at Haddassah Medical School, Jerusalem. In the past problems have been encountered by many workers in the transfer of Leishmania cultures from Jerusalem. Recent experience has shown that the poor viability of cultures previously received from Jerusalem has been due to a toxic substance in the serum vials normally used by the Jerusalem staff for transporting Leishmania cultures. When alternative containers were used all cultures arrived in a viable state. Some difficulty has also been encountered in the transfer of Honduran isolates from the Walter Reed Army Institute of Research, Washington.

Annex I lists Leishmania isolates in the Liverpool reference collection.

1.1.2 Cultivation

We have recently been investigating the growth of Leishmania promastigotes in the defined medium of Steiger and Steiger (J. Parasit., 62, 1010-1011, 1977). Such a medium offers considerable potential for the study of metabolism of Leishmania, and could also ensure the production of reproducible antigens for use in immunological studies. The results so far appear very promising and this medium seems to be capable of supporting the growth of a number of species of Leishmania, including L. aethiopica, L. donovani s. l. and L. mexicana amazonensis. This study will be extended to include other species, e.g. L. braziliensis which are not readily cultured.

1.2 Ultrastructural studies

1.2.1 Noguchi-Adler body

An ultrastructural study of Noguchi-Adler bodies, comparing those produced by promastigotes of an EF producing strain (L. braziliensis braziliensis - LV64) and a non-EF producing strain (L. hertigi - LV43) grown in high and low concentrations of homologous antibody, showed interesting external differences and intracellular structural changes. In the high concentration of antibody (1 in 10), the agglutinated cell body of L. b. braziliensis contained an extensive precipitate which separates individual parasites and occurs inside swollen flagellar pockets. Many cells appeared to be dead and small membranous residues were seen associated with distended flagella and the flagellar pocket opening. The agglutinated bodies formed by cells of L. hertigi (the non-EF producer) had no dense precipitate, though some precipitate was visible in the flagellar reservoirs and as a thin layer between rounded cell forms (Fig. 1). Many internal changes were produced by either the clumping or antibody effects at the cell surface. The lack of cytokinesis resulted in enlarged cell bodies containing, for instance, up to 5 nuclei, 470 subpellicular microtubules in cross-section, two nuclei dividing at the same time both containing spindles and 5 basal bodies associated with a single kinetoplast.

1.2.2 The nucleus of L. hertigi

Further ultrastructural studies of the nucleus of L. hertigi have revealed paracrystalline arrays of ribosome-like particles, situated close to the karyosome (Fig. 2). These particles are arranged in a series of closely apposed helices, 40 nm wide and up to 1 μm long (Fig. 3). Though only seen several times in many thousands of cultured promastigote nuclei examined, their frequency was greatly increased in the nuclei of agglutinated cells in the Adler bodies at high antibody concentration.

Electron microscope autoradiographic studies on the incorporation of uridine and thymidine into the nucleus have so far indicated an interesting distribution of labelled thymidine. In the first study 66% of grains were formed over the peripheral chromatin inside the nuclear membrane, 25% over the central karyosome, and 9% over the nucleoplasm between these two placements of heterochromatin.

1.3 Biochemical taxonomy

1.3.1 African Leishmania

The past year has seen considerable progress in the biochemical taxonomic studies of Drs. Chance and Schnur, and Mr. Thomas. A study of approximately 60 strains from the Ethiopian zoogeographical region of Africa has been completed using seven taxonomic criteria: (a) the enzyme variants of malate dehydrogenase, glucose phosphate isomerase, 6-phosphogluconate dehydrogenase, and glucose-6-phosphate dehydrogenase; (b) the buoyant density of nuclear and kinetoplast DNA; and (c) the serotype of excreted factor. Figs. 4 and 5 show the electrophoretic variation in glucose phosphate isomerase of a wide range of African Leishmania. A summary of our major findings is given here; for clarity they are also tabulated in Annex II. All the parasites isolated from cases of visceral leishmaniasis in Sudan, Kenya, Senegal and Ethiopia are identical in terms of the parameters studies (the systematics of "L. donovani s. l." will be discussed fully later). A large number of isolates from animals implicated as reservoirs of "L. donovani s. l." have been examined. Apart from the Sudanese strains isolated from rodents, i.e. Arvicanthis, Acomys and wild carnivores (Felis serval and Genetta genetta which were incriminated by Hoogstraal and Heyneman (Am. J. Trop. Med. Hyg., 18, 1091-1210, 1969) as reservoirs of visceral leishmaniasis), all other strains isolated from rodents, (Tatera, Xerus, Arvicanthis, in Kenya and Ethiopia and Senegal) and suspected of being reservoirs of the visceral disease in man have proved to be L. tropica, or more properly L. major. We have identified this parasite isolated from man in West Sudan, the Rift Valley of Kenya and Ethiopia, and Senegal as well as of course the Mediterranean Basin and the Middle East. The only strain isolated from possible reservoirs which has proved identical to "L. donovani s . l." was a strain isolated from a dog in Senegal. Strains isolated from Phlebotomus orientalis in Sudan and P. martini in Kenya have also been shown to be "L. donovani s. l.".

The epidemiological features of L. aethiopica which were already well understood were confirmed by this study. We were also able to confirm the presence of L. aethiopica in the Mt. Elgon focus of cutaneous leishmaniasis in Kenya and also show that the hyrax is a reservoir and P. pedifer a vector.

We have been fortunate to have been able to examine isolates from South West Africa (Namibia). The parasites are unlike any others we have examined. While the isolates from man and P. rossi were identical, the hyrax isolates were distinct. This is difficult to explain since P. rossi harbouring a different parasite were isolated at the mouth of the hyrax burrows.

1.3.2 "L. donovani s. l."

For several years the existence of at least two distinctive parasites causing visceral disease in man has been recognised on the basis of our biochemical characterizations.

(a) The most widespread parasite is that found in Africa, the Mediterranean Basin, South America, Middle East, Central Asia and India. Despite the varying epidemiological patterns associated with the disease in these areas we have found no way of distinguishing the parasites, even though we have extended the range of parameters studied to include enzyme variants of alanine aminotransferase, non-specific esterase and acid phosphatase (the last two enzyme types being determined by Dr. F. Ebert of Hamburg University). This would suggest that these parasites only differ from each other in a few gene loci.

Incidentally we have demonstrated that "Oriental sore" in the Mediterranean region may be caused by this parasite.

(b) The other main visceralizing organism which does not appear to be related to the above species is more restricted in its distribution, being limited to a narrow band from Israel to India. It is quite clear from our results that the parasite is a variant of L. tropica (= L. tropica minor).

Presumably one of these species corresponds to L. donovani and the other to L. infantum. Unfortunately since we have shown that both parasites occur in India where L. donovani was first described, we do not yet know to which of them the specific names should be ascribed.

Further isolates are needed from the type locality of L. donovani for comparison with isolates already in our collection.

1.3.3 L. braziliensis

Future studies of biochemical taxonomy will focus on the New World, especially the L. braziliensis complex. In this connection we have examined strains isolated from the Wellcome personnel in Belèm in an attempt to identify the subspecies of L. braziliensis. The only clear means of identification of subspecies of L. braziliensis is by means of the enzyme 6PGDH, though alanine aminotransferase is also useful in this context. The infections of patients K-K. (LV475) and R.W. (LV 476) appear to have been by L. b. guyanensis.

1.3.4 Studies on Leishmania hertigi

Characterization by Mr. Simon Croft of eight strains of L. hertigi, 5 isolated in Panama and 3 isolated in Brazil, has shown that the strains from these two geographic regions should be separated into two subspecies. Similarities between Panamanian and Brazilian strains include the presence of virus-like particles in all, and the formation of large, elongate amastigotes in mouse peritoneal macrophages at 37°C in vitro. The kinetoplast and nuclear DNA buoyant density and the electrophoretic variants of malate dehydroganse also proved to be similar. Finally, it was not possible to detect the production of excreted factor (EF) by cultured promastigotes using the techniques of Schnur, Zuckerman and Greenblatt (Israel J. Med. Sci., 8, 932-942, 1972). Differences found between Brazilian and Panamanian strains included the electrophoretic variants of glucose phosphate isomerase, and glucose-6-phosphate dehydrogenase. In the Noguchi-Adler test, the growth patterns of promastigotes in dilutions of homologous and heterologous antisera produced against one strain of Panamanian, and one of Brazilian L. hertigi, (according to the method of Adler (Adv. Parasit., 2, 35-96, 1964)) also indicated clear qualitative differences between the groups of strains. Other serological differences were shown by gel-diffusion reactions between these antisera and previously typed EF collected from other species of Leishmania; antiserum from the Panamanian strain cross-reacted only with EF type A, and that from the Brazilian strain only with EF type B (according to the serotype classification of Schnur et al. (loc.cit.).

1.4 The interaction between Leishmania and macrophages in vitro

We have reported previously that Leishmania do not appear to inhibit the fusion of lysosomes with the autophagic vacuole. The amastigote might survive in the autophagic vacuole because in some way the digestive enzymes discharged into the vacuole are inhibited. This possibility has been examined further by determining the acid phosphatase activity within infected macrophages at both the light and electron microscope levels. It is clear that, although acid phosphatase activity can be seen both within the macrophage and the amastigotes, there is almost no activity within the vacuole itself. The activity of enzymes ingested from the culture medium along with amastigotes has been examined by monitoring the activity of horseradish peroxidase introduced into the autophagic vacuole in this manner. The activity of the enzyme was markedly reduced, indicating that the amastigote is indeed capable of inhibiting enzyme function within the autophagic vacuole.

1.5 Immunological studies

1.5.1 The effect of immunopotentiating and immunosuppressive drugs

The development of lesions in TFW mice caused by L. m. amazonensis LV79 was examined when the mice were treated with niridazole, cyclophosphamide and levamisole. The immunosuppressive drugs niridazole and cyclophosphamide produce an initial delay in the appearance of lesions followed by a delay or an inhibition of the self-cure process.

Levamisole, an immunopotentiating agent has the paradoxical effect of enhancing the early development of lesions. This could be explained either by its causing increased phagocytosis or, possibly, by an increase in the size of macrophage populations.

1.5.2 Immunological status of laboratory workers

A survey of members of the Department has been undertaken to establish whether any sensitization to leishmanial antigens has taken place during their work in the laboratory. This has been done using the Migration Inhibition Factor (MIF) test. The results have not been clear cut. Three positive controls: one case of active visceral infection, one active cutaneous infection and one healed cutaneous infection apparently did not recognise the antigen, i.e. no MIF was produced. However, two subjects with considerable exposure to Leishmania in the field but no known disease showed migration inhibition values of 40%. It is hoped to extend these studies to include larger numbers of positive controls, and to ascertain the correlation between lymphocyte blast transformation and the leishmanin skin test (delayed hypersensitivity).

1.6 Metabolic studies

Mr. Reginaldo Brazil has started a study of the metabolism of amastigotes of Leishmania by looking for electrophoretically demonstrable enzymes from experimentally infected macrophages. A comparison is also made with cultured promastigotes in order to establish what metabolic changes occur during the promastigote-amastigote transformation. Thirteen enzymes from a number of metabolic pathways have been examined though some technical difficulties have prevented clear conclusions being drawn yet. Malate dehydrogenase is clearly present in amastigotes and probably unchanged from the enzyme found in promastigotes. There have also been indications that iso-citrate dehydrogenase is present in amastigotes. Thus two Krebs cycle enzymes have been demonstrated in amastigotes. Enzymes of the Krebs cycle also occur of course in promastigotes. The glycolytic enzyme glucose phosphate isomerase has been tentatively identified in amastigotes. Attempts are being made to examine whether the culture forms that are morphologically similar to amastigotes and which can be readily produced by elevated temperatures, are metabolically identical to amastigotes.

1.7 Chemotherapy

Ultrastructural investigations on the site of action of sodium stibogluconate have been carried out using a therapeutic dose of Pentostam (400 mg base/kg). Preliminary investigations on the mode of action of sodium stibogluconate were noted briefly in the 5th Semi-Annual Report. A reduction in ribosome numbers appears to be a primary target of drug action at this high dose. The effect is seen in Fig . 6

Note that most parasites appear normal. There are few intermediates between those of normal ultrastructure and those terminally damaged. Fig. 7 shows a number of parasites from control liver samples. In these are seen membrane bound structures containing membranous material and small dense granules, within the host cell cytoplasm and in the close proximity of the parasites. The parasites lie within a membrane of host cell origin, which is tightly apposed to the parasite's own membrane, and can be detected only at a high magnification.

The "membranous complex" in the host cytoplasm, although not histochemically characterised, is typical of secondary lysosomes formed as a result of autophagic activity. These fuse with the phagosomal membrane surrounding the parasites to form a vacuole. In Fig. 8 is seen debris from a membranous structure within the parasitophorous vacuole.

Further data on the correlation between the action of various drugs in tissue culture and in vivo are being analysed at the time of writing. See attached papers by Lewis and Peters (1977) and Lewis and Besso (1977).

1.8 Field studies

No field work has been possible during the year following Dr. Ashford's departure, on secondment to the University of Papua New Guinea. It is intended however to extend the collaboration with Professor Rioux and Dr. Killick-Kendrick in the Montpellier area, to follow up work initatied in Libya, and extend collaboration with workers in Kenya. ELISA tests are being carried out in association with Dr. Mutinga and his associates on sera from kala-azar foci in West Pokot and the Muchakos area of Kenya.

2. RESEARCH PLANS

As indicated in the Introduction, these have been submitted to WRAIR with our application for a Research Contract. In summary they are:

i) to study the mode of action of existing antileishmanial agents
ii) to evaluate potential new drugs against Leishmania
iii) to develop new chemotherapy models with special reference to New World Leishmania.

We have already received and are studying new drugs from WRAIR. In addition work has started, in collaboration with Dr. R. New of the Department of Biochemistry, Liverpool University, on the incorporation of organic antimonials into liposomes. The interaction of non-specific immunological stimuli with specific chemotherapy is another important line that will be pursued with the collaboration of Dr. A. Griffiths.

We have still far to go in comparing the baseline response of different species and different isolates of identical species, to specific antileishmanial drugs, in order to evaluate actual or potential drug resistance in Leishmania infections. It is also important to seek ways of potentiating drug action by the use of suitable drug combinations, a logical step in the light of the paucity of effective agents at the present time.

In parallel with our chemotherapy studies we will continue (with other support) studies on the following aspects:-

(a) Systematics and zoogeography.

The emphasis this year will be to complete our analysis of material from the New World. Numerous isolates including many from Drs. Lainson and Shaw are being grown in culture in preparation for DNA and enzyme characterisation. New antisera will be prepared for EF analysis, using New World isolates as standards, rather than only those from Old World material.

(b) Parasite biology and biochemistry.

New methods of parasite cultivation will be explored particularly with a view to providing defined media that can be used for our extended studies of parasite biochemistry. Special emphasis will still be given to the more difficult New World isolates of the L. braziliensis complex.

(c) Vaccination.

If personnel, time and funding permit we will pursue the question of active immunisation against Leishmania.

3. CONFERENCES, PUBLICATIONS

3.1 Conferences attended

Professor Peters attended the Joint Meeting of the American Society of Tropical Medicine and Hygiene and the Royal Society of Tropical Medicine and Hygiene, held in Philadelphia, 3rd-5th November, 1976

Professor Peters attended the First National Congress of Parasitology held by the Indian Society of Parasitology in Baroda, 24-26th February, 1977

Professor Peters and Dr. Homewood attended a WHO Workshop on the Biology of Malaria Parasites and on in vitro cultivation, held in New York, 7-12th March, 1977.

Professor Peters attended the Swiss Society of Tropical Medicine Spring Meeting held in Lindau, West Germany, 24-26th March, 1977.

Professor Peters and seven members of the Department attended the Royal Society of Tropical Medicine and Hygiene Laboratory Meeting held in Liverpool, 18th November, 1976

3.2 Publications

Papers published since 5th Semi-Annual Report

Ashford, R. W., Schnur, L. F., Chance, M. L., Samaan, S. A. and Ahmed, H. N. (1977). Cutaneous leishmaniasis in the Libyan Arab Republic. Preliminary ecological findings. Ann. trop. Med. Parasit., 71, 265-271.

Chance, M. L., Schnur, L. F. and Thomas, S. C. (1977). The identity of African rodent Leishmanias. Trans. R. Soc. trop. Med. Hyg., 71, 113.

Chance, M. L. (1976). DNA relationships in the genus Leishmania. In: "Biochemistry of Parasites and Host-parasite Relationships", (ed. Van den Bossche, H.), Elsevier/North-Holland: Amsterdam.

Croft, S. L. and Schnur, L. F. (1977). A comparative morphological study of the Noguchi-Adler phenonomenon. Trans. R. Soc. trop. Med. Hyg., 71, 114.

Croft, S. L., Schnur, L. F. and Chance, M. L. (1977). Characterization of strains of Leishmania hertigi. Trans. R. Soc. trop. Med. Hyg., 71, 113.

Gardener, P. J., Schory, L. and Chance, M. L. (1977). Species differentiation in the genus Leishmania by morphometric studies with the electron microscope. Ann. trop. Med. Parasit., 71, 147-155.

Hommel, M. (1977). New approaches to the experimental study of leishmaniasis. Ph.D. thesis: Liverpool.

Killick-Kendrick, R., Leaney, A. J., Ready, P. D. and Molyneux, D. H. (1977). Leishmania in phlebotomid sandflies. IV. The transmission of Leishmania mexicana amazonensis to hamsters by the bite of experimentally infected Lutzomyia longipalpis. Proc. R. Soc. Lond. B., 196, 105-115.

Lewis, D. H. (1976). Host-parasite interactions of Leishmania in vitro. Ph.D. Thesis: Liverpool.

*Lewis, D. H. and Besso, A. (1977). Enzyme activity within Leishmania parasitophorous vacuoles. Trans. R. Soc. trop. Med. Hyg., 71, 113.

*Lewis, D. H. and Peters, W. (1977). The resistance of intracellular Leishmania parasites to digestion by lysosomal enzymes. Ann. trop. Med. Parasit., 71, 295-310.

Peters, W. (1976). The search for antileishmanial agents. In: "Biochemistry of Parasites and Host-parasite Relationships" (ed. Van den Bossche, H.), Elsevier/North-Holland: Amsterdam, 523-535.

Peters, W. (1977). The diagnosis and treatment of leishmaniasis. Proceedings of the German Tropical Medicine Society Meeting held in Lindau, March 1977, p. 36.

Schnur, L. F. (1976). Leishmanial excreted factors (EF) as skin test reagents in the diagnosis of leishmaniasis. Parasitology, 73, ix.

Schnur, L. F. and Zuckerman, A. (1977). Leishmanial excreted factor (EF) serotypes in Sudan, Kenya and Ethiopia. Ann. trop. Med. Parasit., 71, 273-294.

Papers submitted for publication

Ashford, R. W. (1977). The comparative ecology of Leishmania aethiopica. Bull. CNRS.

Brazil, R. P. (1977). On the identity of Brazilian Leishmania strains and the use of the name L. braziliensis. Trans. R. Soc. trop. Med. Hyg. (correspondence).

Chance, M. L. and Peters, W. (1977). The characterisation and significance of DNA and enzyme variation in the genus Leishmania. Paper to be presented at Fifth Int. Congress of Protozoology, New York, July 1977. J. Protozool.

Chance, M. L., Gardener, P. J. and Peters, W. (1977). The biochemical taxonomy of Leishmania as an ecological tool. Bull. CNRS.

Croft, S. L. (1977). Ultrastructural aspects of the nucleus of Leishmania hertigi. J. Protozool.

Killick-Kendrick, R., Molyneux, D. H., Hommel, M., Leaney, A. and Robertson, E. (1977). Leishmania in phlebotomid sandflies, V. The nature and significance of infections of the pylorus and ileum of the sandfly by leishmaniae of the braziliensis group. Proc. R. Soc. Lond. B.

Molyneux, D. H., Lewis, D. H. and Killick-Kendrick, R. (1977). Aspects of the microecology of Leishmania. Bull. CNRS.

Peters, W., Chance, M. L., Mutinga, M. J., Ngoka, J. M. and Schnur, L. F. (1977). The identification of human and animal isolates of Leishmania from Kenya. Ann. trop. Med. Parasit.

Schnur, L. F. (1977). Insect flagellate excreted factors from leptomonad, crithidial and herpetomonad strains. J. Protozool.

4. APPENDICES

Annex I	List of isolates in reference collection	19 pages
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Figure 3	*L. hertigi* showing helical arrangement of ribosome-like particles
Figure 4	Enzyme variants of glucose phosphate isomerase
Figure 5	Enzyme variants of glucose phosphate isomerase
Figure 6	Effect of a single subcutaneous dose of Pentostam on "*L. donovani* s. l."
Figure 7	Untreated "*L. donovani* s. l." infection in mouse liver
Figure 8	Untreated "*L. donovani* s.l." infection in mouse liver showing debris of membranous structure

Publications by Lewis and Peters (1977) and Lewis and Besso (1977)

ANNEX I

List of isolates maintained in the reference collection

Key to donors

1	Dr. A. A. Herrer, Balboa Heights, Panama Canal Zone
2	Drs. R. Lainson and J. J. Shaw, Belem, Brazil
3	Professor W. H. R. Lumsden, London School of Hygiene and Tropical Medicine
4	Professor A. Zuckerman, WHO International Reference Centre for Leishmaniasis, Jerusalem, Israel
5	Hoffman La Roche, Basle, Switzerland
6	Professor F. Pifano, Caracas, Venezuela
7	Professor J. W. Torrealba, Valencia, Venezuela
8	Dr. J. Convit, Caracas, Venezuela
9	Professor D. Bradley, Ross Institute, London School of Hygiene and Tropical Medicine
10	Professor W. Barbosa, Goiania, Brazil
12	Colonel B. C. Walton, Balboa Heights, Panama Canal Zone
13	Dr. R. Neal, Beckenham, England
14	Professor J. A. Rioux, Montpellier, France
15	Professor W. Mayrink, Belo Horizonte, Brazil
16	Liverpool School of Tropical Medicine
17	Professor J. Ranque, Marseille, France
18	Dr. R. Zeledon, San Jose, Costa Rica
19	Professor J. J. Laarman, Instituut voor Tropische Hygiene, Amsterdam
20	Professor R. Bray, Fajara, The Gambia
21	Professor V. Saf'janova, Gameleya Institute, Moscow
22	Dr. M. J. Mutinga, Nairobi, Kenya
23	Professor M. La Placa, Universita di Bologna, Italy
24	Dr. R. P. Brazil, Rio de Janeiro, Brazil
25	Dr. R. E. Abdalla, Khartoum, Sudan
26	Dr. M. Hommel, Liverpool School of Tropical Medicine
27	Dr. R. W. Ashford, Liverpool School of Tropical Medicine
28	Dr. Radim, University of Teheran, Iran
29	Dr. M. Sharma, National Institute of Communicable Diseases, New Delhi, India
30	Professor H. Muhlpfordt, Bernhard-Nocht Institut, Hamburg, Germany
31	Dr. N. Mansour, NAMRU-3, Cairo, Egypt
32	Dr. E. Tikasingh, Caribbean Epidemiology Centre, Trinidad
33	Dr. Grové, South African Institute of Medical Research, Johannesburg
34	Dr. A. Lemma, Addis Ababa, Ethiopia
35	Professor G. Piekarski, Institute of Medical Parasitology, Bonn, W. Germany
36	Dr. J. Baker, Molteno Institute, Cambridge
37	Professor P. C. C. Garnham, Imperial College Field Station, Ascot, England
38	Dr. P. Rees, University of Nairobi, Kenya
39	Captain L. Quigg, Brooke Army Medical Center, Texas, USA
40	Dr. J. Dedet, Institut Pasteur d'Algerie, Algeria.
41	Dr. A. Bryceson, Hospital for Tropical Diseases, London
42	Dr. D. Weinman, Yale University, New Haven, USA
43	Dr. D. H. Molyneux, Liverpool School of Tropical Medicine
44	Miss N. Lupton, Liverpool School of Tropical Medicine
45	Dr. A Ebrahimzadeh, Jondi Shapur University, Iran
46	
47	Dr. F. Sukkar, Ministry of Health, Baghdad
48	Dr. F. Neva, National Institutes of Health, Bethesda, USA.

Key to abbreviations used in notes

A	amastigotes available
CL	cutaneous lesion
DCL	disseminated cutaneous leishmaniasis
KA	kala-azar
LR	leishmaniasis recidivans
LRC	leishmaniasis reference collection (Jerusalem)
MCL	multiple cutaneous lesions
MUC	mucocutaneous
PKDL	post kala-azar dermal leishmanoid
SCL	single cutaneous lesion
V	visceral
NS	normal skin
lump	London University Medical Parasitology
T	trypanosome

LV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV1	L. aethiopica	L86	Bray (20)	Ethiopia	Man	CL
LV2	L. tropica minor	L69	Neal (13)	USSR	?	
LV3	L. m. amazonensis	L15	Lumsden (3)	Brazil, Belem	Man	DCL
LV4	L. m. mexicana	M379, L11	Lumsden (3)	British Hondruas	Nyctomys sumichrasti	A
LV5	? L. enriettii	L88	Lumsden (3)	Brazil	Cavia porcellus	
LV6	? L. enriettii		Zuckerman (4)	Brazil	Cavia procellus	
LV7	? L. enriettii		Zuckerman (4)	Brazil	Cavia procellus	
LV8	L. tropica major	P	Neal (13)	USSR	Rodent	= LV39
LV9	L. donovani	L82, HV3	Lumsden (3)	Ethiopia, Humera	Man	KA, A
LV10	L. m. amazonensis	PH8	Lainson & Shaw (2)	Brazil	Lutzomyia flaviscutellata	A = LV223
LV11	L. m. amazonensis	M649	Lainson & Shaw (2)	Brazil	Proechimys guyanensis	A, SCL, tail = LV75
LV12	? L. donovani chagasi	M884	Lainson & Shaw (2)	Brazil	Cerdocyon thous	V, kidney, A
LV13	L. aethiopica	L96	Bray (20)	Ethiopia	Man	CL
LV14	L. aethiopica	L102	Bray (20)	Ethiopia	Man	DCL
LV15	L. aethiopica	L97	Bray (20)	Ethiopia	Hyrax	
LV16	L. aethiopica	5565	Bray (20)	Ethiopia, Kutaber	Phlebotomus longipes	
LV17	L. aethiopica	5467	Bray (20)	Ethiopia, Kutaber	P. longipes	
LV18	L. aethiopica	5492	Bray (20)	Ethiopia, Kutaber	P. longipes	
LV19	L. b. guyanensis	M1176	Lainson & Shaw (2)	Brazil	Man	A. MCL = LV227
LV20	L. b. braziliensis	M904	Lainson & Shaw (2)	Brazil	Man	MUC, A
LV21	L. sp.	M1411	Lainson & Shaw (2)	Brazil, Marajo	Choloepus didactylus	V, A
LV22	L. mexicana		La Roche (5)			Coelho
LV23	L. donovani	Fulton	STM (16)		Man	KA, A
LV24	L. aethiopica	L100	Bray (20)	Ethiopia, Addis	Man	DCL
LV25	L. aethiopica	L111	Bray (20)	Ethiopia, Kutaber	Hyrax	
LV26	L. aethiopica	L122	Bray (20)	Ethiopia, Kutaber	P. longipes	
LV27	L. aethiopica	L123	Bray (20)	Ethiopia, Kutaber	P. longipes	
LV28	L. aethiopica	L124	Bray (20)	Ethiopia, Kutaber	P. longipes	
LV29	L. aethiopica	L127	Bray (20)	Ethiopia, Ochollo	Man	CL
LV30	L. adleri	1433	Lumsden (3)	Kenya	Hamster (expt. inoc.)	= LV34 LUMP 701
LV31	L. hoogstraali	NG26	Lumsden (3)	Sudan	Hemidactylus turcicus	LUMP 702 LV60
LV32	L. agamae	Agamae 27	Lumsden (3)	Israel	Agama stellio	LUMP 703
LV33	L. adleri	SKINK 7	Lumsden (3)	Kenya	Latastia longicauda	LUMP 704
LV34	L. adleri	146	Lumsden (3)	Kenya	L. l. revoili	LUMP 705
LV35	L. sp.	(87)	Lumsden (3)	Malta or Italy	Tarentola sp.	LUMP 706

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV38	L. infantum	L53	Lumsden (3)	Cyprus	Man	
LV39	L. tropica major	P	Neal (13)	USSR	Rodent	= LV8
LV40	L. mexicana	1235	Herrer (1)	Panama	Proechimys semispinosus	A
LV41	L. mexicana	1746	Herrer (1)	Panama	Oryzomys capito	A
LV42	L. hertigi	C8	Herrer (1)	Panama	Coendou rothschildi	
LV43	L. hertigi	C119	Herrer (1)	Panama	Coendou rothschildi	
LV44	L. braziliensis	LS94	Herrer (1)	Panama	Man	
LV45	L. braziliensis	LS170	Herrer (1)	Panama	Man	
LV46	L. braziliensis	D35	Herrer (1)	Panama	Dog	
LV47	L. braziliensis	D268	Herrer (1)	Panama	Dog	
LV48	L. braziliensis	755	Herrer (1)	Panama	Choloepus hoffmanni	
LV49	L. braziliensis	3146-3	Herrer (1)	Panama	C. hoffmanni	
LV50	L. braziliensis	593-1	Herrer (1)	Panama	Bradypus infuscatus	
LV51	L. braziliensis	2909-5	Herrer (1)	Panama	C. hoffmanni	
LV52	L. braziliensis	M52	Herrer (1)	Panama	Saguinus geoffreyi	
LV54	L. braziliensis	LS127	Herrer (1)	Peru	Man	MUC
LV						
LV56	Trypanosoma rangeli	3026-5	Herrer (1)	Panama	Choloepus hoffmanni	
LV57	T. rangeli	3033-6	Herrer (1)	Panama	C. hoffmanni	
LV58	Endotrypanum schaudinni	415	Herrer (1)	Panama	Bradypus infuscatus	
LV59	E. schaudinni	1222	Herrer (1)	Panama	C. hoffmanni	
LV60	L. hoogstraali	NG26	Lainson & Shaw (2)	Sudan	Lizard	= LV31
LV61	? L. donovani chagasi	M883	Lainson & Shaw (2)	Brazil	Cerdocyon thous	
LV62	L. b. braziliensis	H8	Lainson & Shaw (2)	Brazil	Man	SCL, A
LV63	L. b. braziliensis	H9	Lainson & Shaw (2)	Brazil	Man	SCL, A
LV64	L. b. braziliensis	M1287	Lainson & Shaw (2)	Brazil	Man	MUC, A
LV65	L. b. braziliensis	M1670	Lainson & Shaw (2)	Brazil	Man	MUC, A
LV66	L. b. guyanensis	H1	Lainson & Shaw (2)	Brazil	Man	CL, A
LV67	L. b. guyanensis	M1113	Lainson & Shaw (2)	Brazil	Man	CL, A
LV68	L. b. guyanensis	M 1133	Lainson & Shaw (2)	Brazil	Man	CL, A
LV69	L. b. guyanensis	M1142	Lainson & Shaw (2)	Brazil	Man	CL, A
LV70	L. sp.	M1580	Lainson & Shaw (2)	Brazil	Proechimys guayannensis	V, A
LV71	L. sp.	M1597	Lainson & Shaw (2)	Brazil	Didelphis marsupialis	V
LV72	L. m. amazonensis	H6	Lainson & Shaw (2)	Brazil	Man	SCL
LV73	L. m. amazonensis	M1253	Lainson & Shaw (2)	Brazil	Man	SCL
LV74	L. m. amazonensis	M655	Lainson & Shaw (2)	Brazil	Proechimys sp.	SCL, tail
LV75	L. m. amazonensis	M649	Lainson & Shaw (2)	Brazil	Proechimys guyannensis	A, SCL, tail =LV13

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV76	L. m. amazonensis	M22	Lainson & Shaw (2)	Brazil	Oryzomys capito	SCL, tail
LV77	L. m. amazonensis	M33	Lainson & Shaw (2)	Brazil	Oryzomys capito	SCL, tail
LV78	L. m. amazonensis	M1845	Lainson & Shaw (2)	Brazil	Proechimys sp.	A, NS
LV79	L. m. amazonensis	M1841	Lainson & Shaw (2)	Brazil	Proechimys sp.	NS, A
LV80	L. m. amazonensis	M1830	Lainson & Shaw (2)	Brazil	Metachirus sp.	NS, lost
LV81	L. m. amazonensis	M1824	Lainson & Shaw (2)	Brazil	Oryzomys capito	NS, A
LV82	L. m. amazonensis	M832	Lainson & Shaw (2)	Brazil	Proechimys sp.	SCL, tail
LV83	L. m. amazonensis	M1132	Lainson & Shaw (2)	Brazil	Man	DCL
LV85	E. schaudinni	M1104	Lainson & Shaw (2)	Brazil (Utinga)	C. didactylus	
LV86	E. schaudinni	M1993A	Lainson & Shaw (2)	Brazil, Marajo	C. didactylus	
LV87	E. schaudinni	F907	Lainson & Shaw (2)	Panama, Achlote	C. hoffmanni	
LV88	E. monterogeii	A9	Lainson & Shaw (2)	Costa Rica, San Jose	C. hoffmanni	
LV89	Endotrypanum sp.	Ph26	Lainson & Shaw (2)	Panama	Lutzomyia trapidoi	
LV90	L. enriettii		Bradley (9)	Brazil	Cavia porcellus	A
LV91	L. pifanoi	Gladys	Pifano (6)	Venezuela	Man, Gladys Castillo	DCL
LV						
LV93	? L. donovani	Gladys	Pifano (6)	Venezuela	Man	
LV94	? L. pifanoi	Oecomys	Pifano (6)	Venezuela	O. concolor	
LV95	? L. pifanoi	LL1	Pifano (6)	Venezuela	Man	DCL
LV96	L. pifanoi	LL1	Pifano (6)	Venezuela	Man Rodrigues	= LV135, DCL, hamster, A.
LV97	? L. braziliensis	LBG	Pifano (6)	Venezuela	Man	
LV98	? L. peruviana		Pifano (6)	Peru	Man	uta
LV103	L. braziliensis	AD	Torrealba (7)	Venezuela	Man, A. Duerte	A.
LV104	L. braziliensis	IB	Torrealba (7)	Venezuela	Man, I. Blanco	A
LV105	L. pifanoi	JRC	Convit (8)	Venezuela	Man	DCL, A
LV						
LV108	L. tarentolae	G10	Ranque (17)	Senegal	Gecko	
LV109	L. sp.	RV24	Ranque (17)	Senegal	Arvicanthis	
LV110	L. sp.	BO-DK	Ranque (17)	Senegal	Man	CL
LV111	L. sp.	C3	Ranque (17)	Senegal	Dog	
LV112	L. tropica	Huot LTH	Ranque (17)	France, Provence	Man	CL, lost
LV113	L. sp.	LKA Phil	Ranque (17)	France, Provence	Dog	
LV114	L. infantum	KA ♂	Ranque (17)	France, Provence	Man	KA

LIV No.	Species	Isolate No	Donor	Where isolated	Source	Notes
LV115	Crithidia oncopelti	SO	Ranque (17)			
LV116	C. fasciculata	SF	Ranque (17)			
LV117	Leptomonas ctenocephali	LC	Ranque (17)			
LV						
LV119	L. sp.	M4	Barbosa (10)	Brazil, Mato Grosso	Man	CL, Sao Antonio
LV						
LV121	L. donovani	45	Mayrink (15)	Brazil, Minas Gerais	Man	
LV122	L. donovani	46	Mayrink (15)	Brazil, Minas Gerais	Man	
LV123	L. donovani	50	Mayrink (15)	Brazil, Minas Gerais	Dog	
LV						
LV125	L. donovani	L112	Zuckerman (4)	India	Man	LRC-L155, KA
LV126	L. donovani	L113	Zuckerman (4)	India	Man	Ka, LRC-L156
LV127	L. donovani	L114	Zuckerman (4)	India	Man	KA, LRC-L157
LV128	L. donovani	L115	Zuckerman (4)	India	Man	KA, LRC-L158
LV129	L. donovani	L116	Zuckerman (4)	India	Man	PKDL, LRC-L159
LV130	L. donovani	L117	Zuckerman (4)	India	Man	PKDL, LRC-L160
LV131	L. donovani	L118	Zuckerman (4)	India	Man	PKDL, LRC-L161
LV132	L. donovani	L119	Zuckerman (4)	India	Man	PKDL, LRC-L162
LV						
LV134	L. braziliensis	LRC-L86	Zuckerman (4)	Peru	Man	MUC
LV135	L. sp.	LL1	Zuckerman (4)	Venezuela	Man	=LV96, DCL, LRC-L90
LV136	L. donovani	Brazil 8	Zuckerman (4)	Brazil, Minas Gerais	Man	LRC-L55
LV137	L. donovani		Zuckerman (4)	Kenya	Man	new strain LRC-L58
LV138	L. donovani	VP74	Zuckerman (4)	Sudan	Man	LRC-L61
LV139	L. donovani		Zuckerman (4)	Sudan	Man	Sudan 1 LRC-L66, CL
LV140	L. infantum	Abu-Ghosh 123	Zuckerman (4)	Israel	Man	LRC-L43
LV141	L. tropica	LRC-L34	Zuckerman (4)	Israel (Negev)	Man	
LV142	L. tropica	LRC-L32	Zuckerman (4)	Iraq	Man	LR
LV143	L. tropica	LRC-L35	Zuckerman (4)	Iran (Teheran)	Man	
LV144	L. sp.	Vega	Zuckerman (4)	Panama	Man	LRC-L99
LV145	L. b. panamensis	53A	Walton (12)	Panama	Man, C. Legoas	CL
LV147	L. b. panamensis	56C	Walton (12)	Panama	Man, D. Husbands	
LV148	L. b. panamensis	47A	Walton (12)	Panama	Man, F. Gomez	CL
LV149	L. b. panamensis	42B	Walton (12)	Panama	Man, Saunders	CL
LV150	L. b. panamensis	13	Walton (12)	Panama	Man, Sass	CL
LV151	L. b. panamensis	22	Walton (12)	Panama	Man, J. Burns	CL

LIV No.	Species	Isolate No	Donor	Where isolated	Source	Notes
LV158	L. b. guyanensis	M2061	Lainson & Shaw (2)	Brazil	Man	
LV						
LV160	L. m. amazonensis	M2269	Lainson & Shaw (2)	Brazil, Para	Man	CL
LV161	L. m. amazonensis	M2321	Lainson & Shaw (2)	Brazil	Man	CL
LV162	L. m. amazonensis	M2318	Lainson & Shaw (2)	Brazil	Man	CL
LV163	L. sp.	M2061	Lainson & Shaw (2)	Brazil, Amapa	Man	CI
LV164	L. m. amazonensis	M1384	Lainson & Shaw (2)	Brazil	Dasyprocta sp.	NS
LV165	L. sp.	M2183	Lainson & Shaw (2)	Brazil, Amapa	Man	CL, A
LV166	L. sp.	M2184	Lainson & Shaw (2)	Brazil, Amapa	Man	CL, A
LV167	L. m. amazonensis	M1957	Lainson & Shaw (2)	Brazil	Marmosa murina	NS
LV168	L. m. amazonensis	M839	Lainson & Shaw (2)	Brazil, Mato Grosso	Nectomys squamipes	NS
LV169	L. m. amazonensis	M740	Lainson & Shaw (2)	Brazil, Para	Marmosa murina	CL, tail
LV170	L. m. amazonensis	PH1	Lainson & Shaw (2)	Brazil	Lutzomyia flaviscutellata	
LV171	L. m. amazonensis	PH7	Lainson & Shaw (2)	Brazil	Lutzomyia flaviscutellata	
LV172	L. m. amazonensis	H17	Lainson & Shaw (2)	Brazil	Man	
LV173	L. m. amazonensis	M786	Lainson & Shaw (2)	Brazil, Mato Grosso	Marmosa murina	CL
LV174	L. donovani	L139	Lumsden (3)	Ethiopia, Metemma	Man	KA, A
LV175	L. donovani	L140	Lumsden (3)	Ethiopia, Metemma	Man	KA, A
LV176	L. sp.	6	Zuckerman (4)	Brazil, Ceara	Man	MUC, LRC-L77 = LV222, lost
LV177	L. sp.	CR2, OCR	Zuckerman (4)	Costa Rica	Man	LRC-L75
LV178	L. sp.		Zuckerman (4)	Peru	Man	Uta, LRC-L85
LV179	L. donovani	old strain	Zuckerman (4)	Kenya	Man	LRC-L53
LV181	L. sp.	Ti	Zuckerman (4)	Kenya	Tatera sp.	LRC-L119
LV182	L. sp.	Goedbloed	Laarman (19)	Surinam	Man	CL
LV183	L. sp.	Rijpstra 72	Laarman (19)	Netherlands	Man	CL
LV184	L. sp.	Rijpstra 74	Laarman (19)	Netherlands	Man	CL
LV185	L. sp.	Courtwright	Neal (13)	Panama	Man	CL
LV186	L. sp.	Boynton	Neal (13)	Panama	Man	CL
LV187	L. b. panamensis	Atenzana	Neal (13)	Panama	Man	ex. Bolivia MUC
LV188	L. sp.	Clonts	Neal (13)	Panama	Man	CL
LV189	L. sp.	Baker	Neal (13)	Panama	Man	CL
LV190	L. sp.	L280	Neal (13)	Peru	Man	MUC, uta
LV191	L. sp.	OCRB	Neal (13)	Costa Rica	Man	
LV192	? L. donovani	XI	Rioux (14)	France, Herault	Man	
LV193	? L. donovani	XXVII	Rioux (14)	France, Ardeches	Man	

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV194	? L. donovani	XXXI	Rioux (14)	France, Ardeches	Man	
LV195	? L. donovani	XXXVII	Rioux (14)	France, Herault	Man	
LV196	L. donovani	XXXIX	Rioux (14)	France	Man	
LV197	L. sp.	XXXVI	Rioux (14)	France	Man	
LV199						
LV199	L. sp.	XXV	Rioux (14)	France, Herault	Dog	
LV200	L. sp.	XXXV	Rioux (14)	France, Gard	Dog	= LV208
LV201	L. sp.	XL	Rioux (14)	France, Ardeches	Dog	
LV202	L. sp.	7636	Rioux (14)	France, Corse	Dog	
LV204	L. sp.	Flic	Rioux (14)	France, Corse	Dog	
LV205	L. sp.	Portress	Rioux (14)	France, Herault	Dog	
LV206	L. sp.	Orchidee	Rioux (14)	France, Herault	Dog	
LV207	L. sp.	Jobic	Rioux (14)	France, Gard	Dog	
LV208	L. sp.	Arvic 3878	Rioux (14)	France, Corse	Arvicanthis sp.	= LV200
LV209	L. sp.	1	Convit (8)	Venezuela	Man	CL
LV210	L. sp.	2	Convit (8)	Venezuela	Man	DCL
LV211	L. sp.	P101	Zeledon (18)			
LV212	L. sp.	P202	Zeledon (18)			
LV213	L. sp.	CRO	Zeledon (18)			
LV214	L. sp.	CR100	Zeledon (18)			
LV215	L. sp.	CR200	Zeledon (18)			
LV216	L. sp.	CR300	Zeledon (18)			
LV217	L. sp.	CR400	Zeledon (18)			
LV218	L. sp.	CR500	Zeledon (18)			
LV219	L. sp.	CR600	Zeledon (18)			
LV220	L. sp.	CR700	Zeledon (18)			
LV221	? L. braziliensis	4	Mayrink (15)	Brazil, Bahia	Man	DCL
LV222	? L. b. braziliensis	6	Mayrink (15)	Brazil, Ceara	Man	CL
LV223	L. m. amazonensis	PH8	Mayrink (15)	Brazil, Para	Lutzomyia flaviscutellata	= LV10
LV224	? L. b. braziliensis	49	Mayrink (15)	Brazil, Goias	Man	
LV225	? L. b. braziliensis	10	Mayrink (15)	Brazil, Minas Gerais	Man	
LV226	? L. b. braziliensis	102	Mayrink (15)	Brazil, Minas Gerais	Man	
LV227	L. b. guyanensis	H1176	Mayrink (15)	Brazil, Para	Man	MCL = LV19
LV228	? L. b. braziliensis	121	Mayrink (15)	Brazil, Minas Gerais	Man	
LV229	? L. b. braziliensis	122	Mayrink (15)	Brazil, Minas Gerais	Man	
LV230	? L. b. braziliensis	123	Mayrink (15)	Brazil, Minas Gerais	Man	
LV231	? L. b. braziliensis	124	Mayrink (15)	Brazil, Minas Gerais	Man	

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV232	? L. b. braziliensis	125	Mayrink (15)	Brazil, Minas Gerais	Man	
LV233	? L. b. braziliensis	126	Mayrink (15)	Brazil, Minas Gerais	Man	
LV234	? L. b. braziliensis	127	Mayrink (15)	Brazil, Minas Gerais	Man	
LV235	? L. b. braziliensis	128	Mayrink (15)	Brazil, Minas Gerais	Man	
LV236	? L. b. braziliensis	130	Mayrink (15)	Brazil, Minas Gerais	Man	
LV237	? L. b. braziliensis	131	Mayrink (15)	Brazil, Minas Gerais	Man	
LV238	? L. b. braziliensis	132	Mayrink (15)	Brazil, Minas Gerais	Man	
LV239	? L. b. braziliensis	133	Mayrink (15)	Brazil, Minas Gerais	Man	
LV240	L. donovani	80 MLR	Mayrink (15)	Brazil, Minas Gerais	Man	KA
LV241	L. donovani	60	Mayrink (15)	Brazil, Minas Gerais	Man	KA
LV242	? L. b. braziliensis	120	Mayrink (15)	Brazil, Minas Gerais	Man	
LV243	? L. b. braziliensis	100	Mayrink (15)	Brazil, Minas Gerais	Man	exptl. hamster V.
LV247	L. gymnodactyli	Ag	Saf'janova (21)	USSR, Turkmenia	Agama sanguinolenta	
LV248	L. donovani	VL	Saf'janova (21)	USSR, Turkmenia	Man	KA
LV249	L. tropica minor	IX	Saf'janova (21)	USSR, Azerbaijan	Man	CL
LV250	L. tropica minor	29Askh	Saf'janova (21)	USSR, Turkmenia	Man	CL
LV251	L. tropica minor	32 Askh	Saf'janova (21)	USSR, Turkmenia	Man	CL
LV252	L. tropica major	5Askh	Saf'janova (21)	USSR, Turkmenia	Man	CL
LV253	L. tropica major		Saf'janova (21)	USSR, Turkmenia	Man	CL
LV254	L. tropica major	19Askh	Saf'janova (21)	USSR, Turkmenia	Man	CL
LV255	L. sp.	M2580	Lainson & Shaw (2)	Brazil, Para	Proechimys sp.	V
LV258	L. sp.	M2571	Lainson & Shaw (2)	Brazil, Para	Man	
LV259	L. sp.	M2616	Lainson & Shaw (2)	Brazil, Para	Man	
LV260	L. hertigi	C5	Herrer (1)	Panama	Coendou sp.	
LV261	L. hertigi	C122	Herrer (1)	Panama	Coendou sp.	
LV263	L. braziliensis	1128	Walton (12)	Peru, Amazonia	Man	? MUC
LV264	L. braziliensis	1132	Walton (12)	Honduras	Man	CL
LV265	L. b. panamensis	11311	Walton (12)	Panama	Man	CL
LV266	L. aethiopica	KPS H2	Mutinga (22)	Kenya		
LV267	? L. donovani	BOL 72	La Placa (23)	Italy	Man	? lumph node
LV268	L. m. goulerti	4	Brazil (24)	Brazil, Bahia	Man	DCL
LV269	L. donovani	XI	Rioux (14)	France, Herault	Man	KA = LV192
LV270	L. donovani	XXVII	Rioux (14)	France, Ardeche	Man	KA = LV193
LV271	L. donovani	XXXI	Rioux (14)	France, Aude	Man	KA = LV194
LV272	"L. canis"	XXV	Rioux (14)	France, Herault	Dog	= LV199
LV273	L. tropica	XXXVIII	Rioux (14)	France, Gard	Man	OS = LV198
LV274	L. donovani	XLI	Rioux (14)	France, Gard	Man	KA
LV						

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV275	"L. canis"	Fanfare	Rioux (14)	France	Dog	from hamster 4272
LV276	"L. canis"	Monton	Rioux (14)	France	Dog	
LV277	L. canis	Blacky	Rioux (14)	France	Dog	
LV278	T. pestanai		Rioux (14)	France	Badger	No. 4152
LV279	T. pestanai		Rioux (14)	France	Badger	No. 4295
LV280	L. donovani	B072	La Placa (23)	Italy, B ologna	Man	KA
LV281	L. donovani	25	La Placa (23)	Sudan?	Man	Standers Khartoum strain?
LV282	L. donovani	BH35	La Placa (23)	Brazil	Dog?	
LV283	L. tropica	B2	La Placa (23)	?		
LV284	L. enriettii		La Placa (23)	?		
LV285	"L. braziliensis"	B8	La Placa (23)	Costa Rica?		
LV286	L. donovani		La Placa (23)	India?		
LV287	L. t. minor		La Placa (23)	USSR	Man	urban dry
LV288	L. tropica	LT	La Placa (23)	USA (Nigeria?)	Man	OS
LV289	L. tropica		La Placa (23)	?		
LV290	L. braziliensis		La Placa (23)	Costa Rica		CL
LV291	L. donovani	T	La Placa (23)	Kerim	Gerbil	
LV292	L. donovani	Dog 326	La Placa (23)	Sicily	Dog	V
LV293	L. donovani	Baby 1973	La Placa (23)	Sicily	Man	KA
LV294	L. donovani	Baby 1974	La Placa (23)	Sicily	Man	KA
LV295	L. sp.	405	La Placa (23)	Italy, Bologna	Reptile	V reptile-hamster 405
LV296	L. donovani	433	La Placa (23)	Ethiopia	Man	KA
LV297	L. donovani	462	La Placa (23)	Kenya	Tatera	
LV298	L. infantum	163	La Placa (23)	France	Dog	
LV299	L. infantum	727	La Placa (23)	Cyprus	rodent? Panagista	probably man
LV300	L. tropica	344	La Placa (23)	Israel	Man	
LV301	L. tropica	567	La Placa (23)	USSR	Rhombomys opimus	
LV302	L. tropica	XLII	Rioux (14)	France	Man?	
LV303	L. canis	Smoky	Rioux (14)	France	Dog	Lost
LV304	L. canis	Yota	Rioux (14)	France	Dog	
LV305	L. sp.		Abdalla (25)	Sudan, Darfur	Man	CL
LV306	L. sp.	M2674	Lainson & Shaw (2)	Brazil	Rodent	
LV307	L. sp.	M2746	Lainson & Shaw (2)	Brazil	Rodent	
LV308	Herpetomonas		Hommel (26)	UK, Liverpool	Culicoides variipennis	
LV309	L. sp.	16	Ashford (27)	Libya, Bir Ayyad	Man	CL
LV310	L. sp.	17	Ashford (27)	Libya, Bir Ayyad	Man	MCL
LV311	L. sp.	18	Ashford (27)	Libya, Bir Ayyad	Man	CL
LV312	L. sp.	20	Ashford (27)	Wadi el Mayyat (Haia)	Man	MCL

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV313	L. sp.	21	Ashford (27)	Bu Rilan	Man	MCL
LV314	L. sp.	28	Ashford 9(27)	Bu Rilan	Man	MCL
LV315	L. sp.	30	Ashford (27)	Ar G'aat	Man	MCL
LV316	L. sp.	55	Ashford (27)	Nalut	Man	MCL
LV317	L. sp.	57	Ashford (27)	N.W. Libya	Man	MCL
LV318	L. sp.	66	Ashford (27)	N.W. Libya	Man	MCL
LV319	L. sp.	77	Ashford (27)	Bu Rilan	Man	MCL
LV320	L. sp.	99	Ashford (27)	Kikla	Man	CL
LV321	L. sp.	102	Ashford (27)	Kikla	Man	CL
LV322	L. sp.	112	Ashford (27)	Kikla	Man	MCL
LV323	L. donovani	1	Nadim -(28)	Iran	Man	MCL, LKA
LV324	L. donovani	2	Nadim (28)	Iran	Dog	KA
LV325	L. tropica	3	Nadim (28)	Iran	Man	urban
LV326	L. tropica	4	Nadim (28)	Iran, Isfahan	Man	rural
LV327	L. tropica	5	Nadim (28)	Iran, Khuzestan	Man	
LV328	L. sp.	6	Nadim (28)	Iran, Khuzestan	Dog, via mouse	
LV329	L. sp.	7	Nadim (28)	Iran, Khorassan	Lizard	
LV330	L. sp.	8	Nadim (28)	Iran, Roudbar	Lizard	
LV331	L. sp.	9	Nadim (28)	Iran, Khuzestan	Lizard	
LV332	L. sp.	10	Nadim (28)	Iran, Isfahan	Lizard	
LV333	L. sp.	CR1000	Zeledon (18)			
LV334	L. sp.	CR1002	Zeledon (18)			
LV335	L. sp.	CR1003	Zeledon (18)			
LV336	L. sp.	CR1004	Zeledon (18)			
LV337	L. sp.	CR1005	Zeledon (18)			
LV338	L. sp.	CR1006	Zeledon (18)			
LV339	L. sp.	CR1007	Zeledon (18)			
LV340	L. sp.	CR1008	Zeledon (18)			
LV341	L. sp.	CR1010	Zeledon (18)			
LV342	L. sp.	CR1012	Zeledon (18)			
LV343	L. sp.	CR1013	Zeledon (18)			
LV344	L. sp.	CR1016	Zeledon (18)			
LV345	L. sp.	CR1017	Zeledon (18)			
LV346	L. sp.	HTBM	Sharma (29)	India	Man	
LV347	L. sp.	GTBM	Sharma (29)	India	Gerbil	
LV348	L. sp.	DBKM	Sharma (29)	India	Dog	
LV349	L. sp.	SDTBM	Sharma (29)	India	Phlebotomus sp.	
LV350	?	SCTB	Sharma (29)	India	S. clydei	

LIV No.	Species	Isolate No	Donor	Where isolated	Source	Notes
LV351	L. braziliensis		Muhlpfordt (30)	Brazil, Ceara	Man	MUC
LV352	L. tropica		Muhlpfordt (30)	Israel	Man	
LV353	L. braziliensis		Muhlpfordt (30)	Panama	Man, J. J. Shaw	
LV354	L. braziliensis		Muhlpfordt (30)	Brazil, Para	Man	
LV355	L. tropica	LRC-L23	Muhlpfordt (30)	Israel, Ein Geddi	Man	
LV356	L. t. major	LRC-L38	Muhlpfordt (30)	USSR, Turkestan	Man	
LV357	L. t. minor	LRC-L39	Muhlpfordt (30)	USSR, Turkestan	Man	
LV358	L. d. infantum	LRC-L47	Muhlpfordt (30)	France	Man	KA
LV359	L. donovani	LRC-L51	Muhlpfordt (30)	India	Man	KA
LV360	L. donovani	LRC-L55	Muhlpfordt (30)	Brazil, Minas Gerais	Man	KA
LV361	L. donovani	LRC-L58	Muhlpfordt (30)	Kenya	Man	KA
LV362	L. donovani	LRC-L61	Muhlpfordt (30)	Sudan	Man	
LV363	L, braziliensis	LRC-L77	Muhlpfordt (30)	Brazil, Ceara	Man	
LV364	L. braziliensis	LRC-L90	Muhlpfordt (30)	Venezuela	Man	DCL
LV365	L. mexicana	LRC-L94	Muhlpfordt (30)	British Honduras	Man	
LV366	L. donovani	51	Muhlpfordt (30)	Sudan	Man	KA Ramru 5895
LV367	L. donovani	53	Muhlpfordt (30)	Sudan, Nile province	Man	
LV368	L. braziliensis		Muhlpfordt (30)	Peru, Panguara	Man	Villwock
LV369	L. tropica	11	Muhlpfordt (30)	Saudi Arabia	Man	Stiehl
LV370	L. tropica	III	Muhlpfordt (30)	Iran, Ahwaz	Man	H. Gorko
LV371	L. donovani		Muhlpfordt (30)	Spain, Alicante	Man	KA
LV372	L. sp.	63-282	Mansour (31)	Sudan, Gabek	Genet	
LV373	L. sp.	636-64	Mansour (31)	Sudan, Gabek	P. orientalis	
LV374	L. donovani	VP 74	Mansour (31)	Sudan	Man	KA
LV375	L. sp.	JB	Mansour (31)	Sudan	Man	J. Bayer CL
LV376	L. sp.	Mcl	Mansour (31)	Sudan	Man	E. McConnell CL
LV377	L. aethiopica	L 93	Mansour (31)	Ethiopia	Man	CL
LV378	L. sp.	Str-H085	Mansour (31)	Ethiopia	Lizard	
LV379	L. sp.	SPO-H099	Mansour (31)	Ethiopia	Lizard	
LV380	L. donovani	K2163	Mansour (31)	Ethiopia, Metemma	Man	
LV381	L. m. amazonensis	71-110	Tikasingh (32)	Trinidad	L. flaviscutellata	
LV382	L. m. amazonensis	TRVL 13521	Tikasingh (32)	Trinidad	Marmosa mitis chapmani	

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV383	L.sp.	1	Laarman (19)	Surinam	Man	
LV384	L. sp.	2	Laarman (19)	Surinam	Man	
LV385	L. sp.	3	Laarman (19)	Surinam	Man	
LV386	L. sp.	POS	Grové (33)	SW Africa, Keetmanshoop	Man	
LV387	L. donovani	K2163	Lemma (34)	Ethiopia, Humera	Man KA	
LV388	L. donovani	HO174	Lemma (34)	Ethiopia, Humera	Arvicanthis niloticus	V
LV389	L. sp.	HO145	Lemma (34)	Ethiopia, Humera	Mabuya sp. skink	T
LV390	L. sp.	HO139	Lemma (34)	Ethiopia, Humera	Mabuya sp. skink	T
LV391		L1	Lainson and Shaw (2)	Brazil	Man	
LV392		L20	Lainson and Shaw (2)	Venezuela	Man	
LV393		H21	Lainson and Shaw (2)	Brazil, Belem	Man	
LV394		SH24	Lainson and Shaw (2)	Panama	Man J.J. Shaw	
LV395		M1756	Lainson and Shaw (2)	Brazil	Man	
LV396		M2373	Lainson and Shaw (2)	Brazil	Man	
LV397		M2374	Lainson and Shaw (2)	Brazil	Man	
LV398		M2625	Lainson and Shaw (2)	Brazil	Man	
LV399		M2682	Lainson and Shaw (2)	Brazil	Man	
LV400		M2704	Lainson and Shaw (2)	Brazil	Man	
LV401		M2737	Lainson and Shaw (2)	Brazil	Rat	
LV402	L. hertigi	M2808	Lainson and Shaw (2)	Brazil	Coendu sp.	
LV403		M3050	Lainson and Shaw (2)	Brazil	Man	
LV404	Leptomonas pessoi	M2693	Lainson and Shaw (2)	Brazil		
LV405	L. sp.	L46	Rioux (14)	France, Cevennes	Man OS	
LV406	L. sp.	L53			Dog	
LV407	L. sp.	L57			Dog "Belle"	
LV408	L. sp.	JOF	Brazil (24)	Brazil, Minas Gerais	Man	CL
LV409	L. sp.	JCS	Brazil (24)	Brazil, Amazonas	Man	
LV410	L. sp.	Baptista	Brazil (24)	Brazil	Man	
LV411	L. sp.	JA	Brazil (24)	Brazil, Sao Paulo	Man	
LV412	L. sp.		Brazil (24)	Brazil		
LV413	L. enriettii		Brazil (24)	Brazil	Cavia procellus	
LV414	L. tarentolae		Brazil (24)	Brazil	Simpson's strain	
LV415	L. m. goulati	4	Brazil (24)	Brazil, Bahia		
LV416	L. donovani	Mayer	Brazil (24)	Brazil	Man	KA
LV417	T. rangeli		Hommel (26)	Panama, Chorrera	Man	WMP 977

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV418	T. musculi		Hommel (26)	Sicily	Mouse	Krampitz strain
LV419	T. lewisi		Hommel (26)	England, Herts.	Rat	Old strain
LV420	T. evotomys		Hommel (26)	England, Cheshire	Clethrionomys glareolus	
LV421	T. blanchardi		Hommel (26)	France	Eliomys quercinus	LUMP 841 mouse strain
LV422	T. rabinowitschae		Hommel (26)	France	Cricetus cricetus	LUMP 848 mouse strain
LV423	T. dionisii	P3	Baker (36)	England	P. pipistrellus	
LV424	T. vespertilionis	N6	Baker (36)	England, Essex	Nyctalus noctula	
LV425	L. sp.	253	Grové (33)	Namibia	Procavia capensis	
LV426	L. sp.	265	Grové (33)	Namibia	Procavia capensis	
LV427	L. sp.	J. Brand	Grové (33)	Namibia	Man	MCL
LV428	L. donovani	L59	Rioux (14)	France	Dog	Belle
LV429	L. sp.	CR1018	Zeledon (18)	Costa Rica	Heteromys dermarestianus	
LV430	L. sp.	M4088	Lainson and Shaw (2)	Brazil	sandfly	
LV431	L. sp.	M4111	Lainson and Shaw (2)	Brazil	sandfly	
LV432	L. b. braziliensis	M2904A	Lainson and Shaw (2)	Brazil	Man	
LV433	L. b. braziliensis	M2904B	Lainson and Shaw (2)	Brazil	hamster	
LV434	L. sp.	M1413	Lainson and Shaw (2)	Brazil	sloth	
LV435	L. b. braziliensis	M4035	Lainson and Shaw (2)	Brazil	Man	
LV436	L. b. braziliensis	M2903	Lainson and Shaw (2)	Brazil	Man	
LV437	L. sp.	M1411	Lainson and Shaw (2)	Brazil	sloth	
LV438	L. sp.	M4042	Lainson and Shaw (2)	Brazil	Procupine	
LV439	L. sp.	M4043	Lainson and Shaw (2)	Brazil	Porcupine	
LV440	L. sp.	M4058	Lainson and Shaw (2)	Brazil	Procupine	
LV441	L. sp.	M4059	Lainson and Shaw (2)	Brazil	Porcupine	
LV442	L. sp.	M4060	Lainson and Shaw (2)	Brazil	Procupine	
LV443	L. sp.		Piekarski (35)	Saudi Arabia	Man	
LV444	L. sp.	Van Staden	Grové (33)	Namibia Keetmanshoop	Man	
LV445	L. donovani	902	Lemma (34)	Ethiopia	Arvicanthis	
LV446	L. donovani	Al 1	Dedet (40)	Algeria Alger	Dog	
LV447	L. donovani	Al 2	Dedet (40)	Algeria Alger	Man KA	
LV448	L. donovani	Al 4	Dedet (40)	Algeria Alger	Man CL	
LV449	L. tropica	Al 6	Dedet (40)	Algeria Biskra	Man CL	
LV450	L. tropica	Al 8	Dedet (40)	Algeria Alger	Man CL	
LV451	L. tropica	Al 10	Dedet (40)	Algeria Bou Saada	Man CL	
LV452	L. tropica		Garnham (37)	Iraq, Baghdad	Rattus rattus blood	
LV453	L. sp.	L1	Mutinga (22)	Kenya, West Pokot	Lizard blood	

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV454	L. sp.	L14	Mutinga (22)	Kenya West Pokot	Lizard blood	
LV455	L. sp.	L17	Mutinga (22)	Kenya West Pokot	Lizard blood	
LV456	L. sp.	L18	Mutinga (22)	Kenya West Pokot	Lizard blood	
LV457	L. sp.	L19	Mutinga (22)	Kenya West Pokot	Lizard blood	
LV458	L. sp.	L33	Mutinga (22)	Kenya Baringo	Lizard blood	
LV459	L. sp.	L35	Mutinga (22)	Kenya Baringo	Lizard blood	
LV460	L. sp.	T1	Mutinga (22)	Kenya Baringo	Tatera V.	
LV461	L. donovani	H9	Mutinga (22)	Kenya West Pokot	Man KA	
LV462	L. donovani	H10	Mutinga (22)	Kenya West Pokot	Man KA	
LV463	L. sp.	S1	Mutinga (22)	Kenya West Pokot	Sandfly	
LV464	L. sp.	S2	Mutinga (22)	Kenya West Pokot	Sandfly	
LV465	L. sp.	S3	Mutinga (22)	Kenya West Pokot	Sandfly	
LV466	L. sp.	S4	Mutinga (22)	Kenya West Pokot	Sandfly	
LV467	L. donovani	Cleopas	Rees (38)	Kenya West Pokot	Man John Cleopas KA	KA resistant
LV468	L. sp.	Harris	Quigg (39)	USA Texas	Man	
LV469	L. sp.	GO3-RA	Lemma (34)	Ethiopia, Omo River	Man	CL
LV470	L. sp.	GO3-RA	Lemma (34)	Ethiopia, Omo River	Man	CL
LV471	L. aethiopica	TA6458	Bryceson (41)	Ethiopia, Omo River	Man	CL
LV472	L. sp.	HR3	Mutinga (22)	Kenya	Hyrax	
LV473	L. sp.	F4	Mutinga (22)	Kenya	Sandfly	
LV474	L. d. chagasi	M2682	Lainson and Shaw (2)	Brazil, Bahia	Man	KA
LV475	L. sp.	M4209	Lainson and Shaw (2)	Brazil, Serra dos Carajas	Man	CL RK-K
LV476	L. sp.	M4147	Lainson and Shaw (2)	Brazil, Jari	Man	CL RDW (1)
LV477	L. sp.	M4177	Lainson and Shaw (2)	Brazil, Jari	Man	CL DK
LV478	L. sp.	M4088	Lainson and Shaw (2)	Brazil, Jari	Sandfly	
LV479	L. sp.	M4108	Lainson and Shaw (2)	Brazil, Jari	Sandfly	
LV480	L. sp.	M4111	Lainson and Shaw (2)	Brazil, Jari	Sandfly	
LV481	L. sp.	M4110	Lainson and Shaw (2)	Brazil, Jari	Sandfly	
LV482	L. sp.	M4146	Lainson and Shaw (2)	Brazil, Jari	Man	CL MCS (1)
LV483	L. sp.	M4196	Lainson and Shaw (2)	Brazil, Jari	Fly	Fleb 42
LV484	L. sp.	M4197	Lainson and Shaw (2)	Brazil, Jari	Fly	Fleb 22
LV485	L. sp.	M4190	Lainson and Shaw (2)	Brazil, Serra dos Carajos	Fly	Fleb 41
LV486	L. sp.	M4200	Lainson and Shaw (2)	Brazil, Jari	Man	RDW (2)

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV487	L. sp.	Rossi I	Grové (33)	Namibia	P. rossi	
LV488	L. sp.	Rossi II	Grové (33)	Namibia	P. rossi	
LV489	L. sp.	Rossi IIIa	Grové (33)	Namibia	P. rossi	
LV490	L. sp.	Rossi IIIb	Grové (33)	Namibia	P. rossi	
LV491	L. sp.		Ashford (27)	Libya	Psammomys	
LV492	T. cyclops		Weinman (42)	Malaysia	Primate	
LV493	T. theileri		LSHTM			
LV494	T. corvi		Baker (36)	England	Rook	
LV495	T. enriettii		Molyneux (43)	Nigeria	bird	
LV496	T. rabinowitschae		Hommel (26)	France	Cricetus cricetus	
LV497	Leptomonas sp.		Hommel (26)	England	rabbit flea	
LV498	T. grosi		Lupton (44)	England	Apodemus sylvaticus	
LV499	L. sp.	SL2	Herrer (1)	Peru	Man, Uta	
LV500	L. sp.	SL3	Herrer (1)	Peru	Man, Uta	
LV501	L. sp.	SL5	Herrer (1)	Peru	Man, Uta	
LV502	L. sp.	D8	Herrer (1)	Peru	Dog	
LV503	L. sp.	T46639	Bryceson (41)	Brazil	Man	RK-K
LV504	L. tropica	54	Ebrahimzadeh (45)	Iran Ahwaz	Man	wet
LV505	L. tropica	67	Ebrahimzadeh (45)	Iran Ahwaz	Man	dry
LV506	L. tropica	95	Ebrahimzadeh (45)	Iran Ahwaz	Man	wet
LV507	L. tropica		Golenser (46)	Spain	Man	
LV508	L. tropica	Inman	Sukkar (47)	Iraq	Man	
LV509	L. tropica	Nadia	Sukkar (47)	Iraq	Man	
LV510	L. tropica	Salma	Sukkar (47)	Iraq	Man	
LV511	L. braziliensis	Hopkins	Sukkar (47)	Iraq	Man	
LV512	L. donovani	Sudan				
LV513	L. tropica	Seidman	Neva (48)	Senegal	Man	CL
LV514	L. t. minor	Ackerman	Neva (48)	Asia	Man	CL
LV515	L. donovani	McComb	Neva (48)	Greece	Man	KA
LV516	L. b. guyanensis	M4146	Lainson and Shaw (2)	Brazil Monte Dourado	Man	MCS
LV517	L. b. guyanensis	M4147	Lainson and Shaw (2)	Brazil Monte Dourado	Man	RDW
LV518	L. b. guyanensis	M4200	Lainson and Shaw (2)	Brazil Monte Dourado	Man	RDW(2)
LV519	L. b. guyanensis	M4209	Lainson and Shaw (2)	Brazil Monte Dourado	Man	RK-K
LV520	L. b. guyanensis	M4210	Lainson and Shaw (2)			JJS
LV521	L. b. guyanensis	M4216	Lainson and Shaw (2)	Brazil Monte Dourado	Lu. anduzei	

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV522	L. b. guyanensis	M4217	Lainson and Shaw (2)	Brazil Monte Dourado	Lu. anduzei	
LV523	L. b. guyanensis	M4218	Lainson and Shaw (2)	Brazil Monte Dourado	Lu. anduzei	
LV524	L. b. guyanensis	M4220	Lainson and Shaw (2)	Brazil Monte Dourado	Lu. anduzei	
LV525	L. b. guyanensis	M4228	Lainson and Shaw (2)	Brazil Monte Dourado	Lu. anduzei	
LV526	L. b. guyanensis	M4231	Lainson and Shaw (2)	Brazil Monte Dourado	Lu. anduzei	
LV527	L. b. guyanensis	M4237	Lainson and Shaw (2)		Man	JJS
LV528	L. b. braziliensis	M4238	Lainson and Shaw (2)		Man	RL
LV529	L. b. guyanensis	M4239	Lainson and Shaw (2)	Brazil Monte Dourado	Man	MCS(2)
LV530	L. b. braziliensis	M4250	Lainson and Shaw (2)		Man	RL (2)
LV531	Leptomonas culicidarum	LRC-L129	Zuckerman (4)		Culex pipiens	Noguchi 1927
LV532	Herpetomonas muscidarum	LRC-L130	Zuckerman (4)		Musca domestica	
LV533	L. sp.	LRC-L141	Zuckerman (4)	Israel	chameleon	
LV534	L. donovani	LRC-L56		Sudan	Rattus rattus	F25
LV535	L. donovani	LRC-L57		Kenya	P. martini	
LV536	L. donovani	LRC-L64		Sudan	Arvicanthis	VPRP65
LV537	L. donovani	LRC-L65		Sudan	Acomys	27A
LV538	L. donovani	LRC-L70		Sudan	G. genetta senegalensis	Sudan 4
LV539	L. donovani	LRC-L71		Sudan	Felis serval phillipsi	Sudan 5
LV540	L. donovani	LRC-L72		Sudan	Acomys albigena	
LV541	L. sp.	LRC-L111		France	Vulpes vulpes	SXXI
LV542	L. sp.	LRC-L116		Kenya	spermophil	
LV543	L. sp.	LRC-L117		Kenya	Tatera	T1
LV544	L. sp.	LRC-L138		Israel	Meriones shawi	
LV545	L. sp.	LRC-L139		Israel	Psammomys	
LV546	L. sp.	LRC-L147		Ethiopia	Man	L100 DCL
LV547	L. sp.	LRC-L149		Ethiopia	Man	L102 DCL
LV548	L. aethiopica	LRC-L164		Ethiopia	Man	L96 OS
LV549	L. sp.	L65	Rioux (14)	France	Dog	
LV550	L. tropica	DK1	Dedet (40)	Senegal	Man	
LV551	L. tropica	DK2	Dedet (40)	Senegal	Man	
LV552	L. tropica	DK3	Dedet (40)	Senegal	Man	
LV553	L. tropica	DK4	Dedet (40)	Senegal	Man	

LIV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV554	L. tropica	LRC L1	Zuckerman (4)	Israel	Man	
LV555	L. tropica	LRC L7	Zuckerman (4)	India	Man	
LV556	L. tropica	LRC L36	Zuckerman (4)	Iraq	Man	= L75
LV557	L. donovani	LRC L52	Zuckerman (4)	India	Man	
LV558	L. donovani	LRC L54	Zuckerman (4)	India	Man	
LV559	L. donovani	LRC L63	Zuckerman (4)	Sudan	P. orientalis	
LV560	L. donovani	LRC L133	Zuckerman (4)	Ethiopia	Man	=HU3
LV561	L. tropica	LRC L137	Zuckerman (4)	Israel	Man	
LV562	L. enriettii	LRC L144	Zuckerman (4)	Brazil	Guinea pig	from Bray
LV563	L. aethiopica	LRC L166	Zuckerman (4)	Kenya	fly	KPS F1
LV564	L. tropica	LRC L4	Zuckerman (4)	Israel, Jericho	fly	
LV565	L. tropica	LRC L18	Zuckerman (4)	Afghanistan		
LV566	L. tropica	LRC L20	Zuckerman (4)	Israel, Negev	Man	
LV567	L. tropica	LRC L22	Zuckerman (4)	Israel, Negev	Man	
LV568	L. tropica	LRC L27	Zuckerman (4)	Israel, Negev	Man	
LV569	L. tropica	LRC L30	Zuckerman (4)	Israel, Negev	Man	
LV570	L. tropica	LRC L33	Zuckerman (4)	Israel, Ein Geddi	Man	
LV571	L. tropica	LRC L34	Zuckerman (4)	Israel, Negev	Man	
LV572	L. infantum	LRC L42	Zuckerman (4)	Israel	Man	
LV573	L. infantum	LRC L44	Zuckerman (4)	Israel	Man	
LV574	L. infantum	LRC L46	Zuckerman (4)	Israel	Man	
LV575	L. donovani	LRC L62	Zuckerman (4)	Sudan	P. orientalis	
LV576	L. tropica	DK7	Dedet (40)	Senegal	Man	
LV577	L. tropica	DK8	Dedet (40)	Senegal	Man	
LV578	L. tropica	DK11	Dedet (40)	Senegal	Man	
LV579	L. tropica	DK21	Dedet (40)	Senegal	Man	
LV580	L. tropica	DK24	Dedet (40)	Senegal	Man	
LV581	L. tropica	DK25	Dedet (40)	Senegal	Man	
LV582	L. sp.	CR1011	Zeledon (18)	Costa Rica	L. trapidoi	
LV583	L. braziliensis	CR1014	Zeledon (18)	Costa Rica	Man	
LV584	L. braziliensis	LASSIE	Zeledon (18)	Costa Rica	Dog	
LV585	L. braziliensis	HSJO-36	Zeledon (18)	Costa Rica	Man	
LV586	L. tropica	Iraq	L.S.T.M. (16)	Iraq	Man	
LV587	L. tropica	Maroc	L.S.T.M. (16)	Marocco	Man	
LV588	L. tropica	Saudi	L.S.T.M. (16)	Saudi Arabia	Man	

ANNEX II

A summary of the hosts and distribution of the species of African Leishmania

		Host	Origin	Notes
1)	L. donovani s.l.	Man	Sudan	Including cutaneous manifestation
		Man	Kenya	
		Man	Ethiopia	
		Arvicanthus	Sudan	Also Ratus and Acomys
		Felis serval	Sudan	Also Genetta
		Dog	Senegal	
		P. orientalis	Sudan	
		P. martini	Kenya	
2)	L. aethiopica	Man	Ethiopia	Including diffuse cutaneous leishmaniasis
		Man	Kenya	
		Hyrax	Ethiopia	
		Hyrax	Kenya	
		P. longipes	Ethiopia	
		P. pedifer	Kenya	
3)	L. major	Man	Sudan	
		Man	Kenya	
		Tatera	Kenya	Isolated from viscera
		Man	Ethiopia	
		Man	Senegal	
		Arvicanthus	Ethiopia	Isolated from viscera
		Arvicanthus	Senegal	Isolated from viscera
		Xerus	Kenya	Isolated from viscera
4)	L. sp.	Man	Namibia	Cutaneous infection
		P. rossi	Namibia	
5)	L. sp.	Hyrax	Namibia	

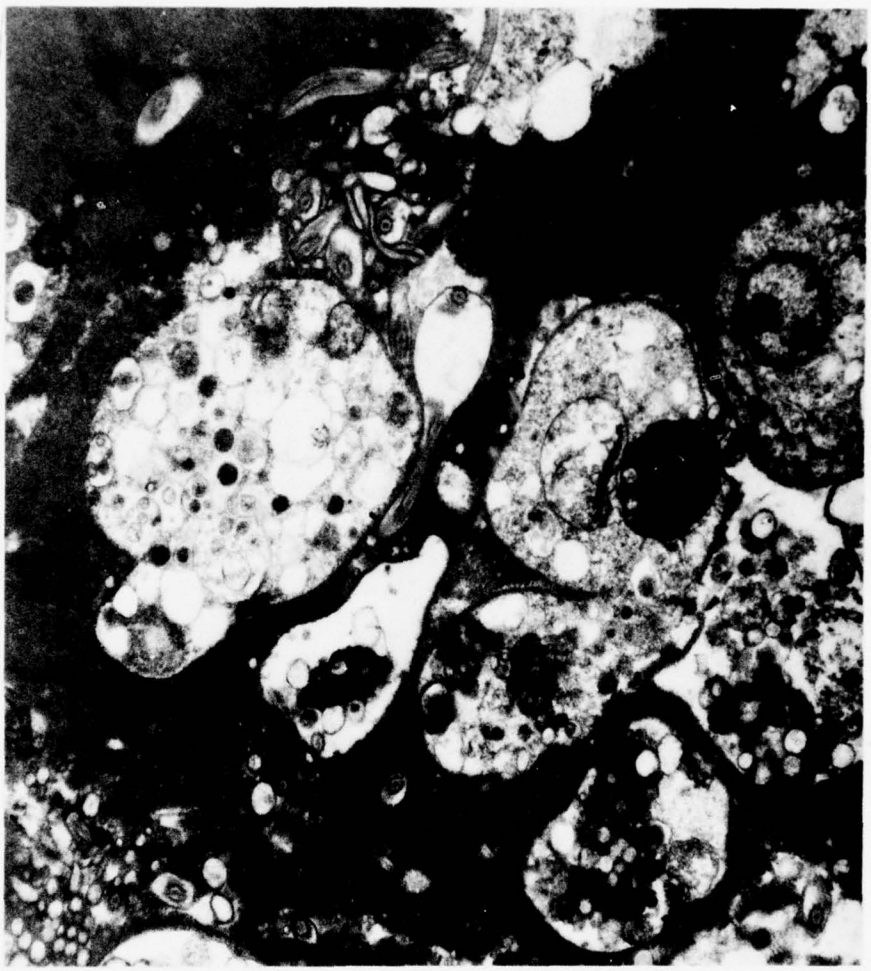

FIGURE 1

L. b. braziliensis in 1:10 dilution of homologous antiserum. Note extensive matrix separating individual parasites, and small membranous vesicles possibly associated with distended flagella.

x 10,000

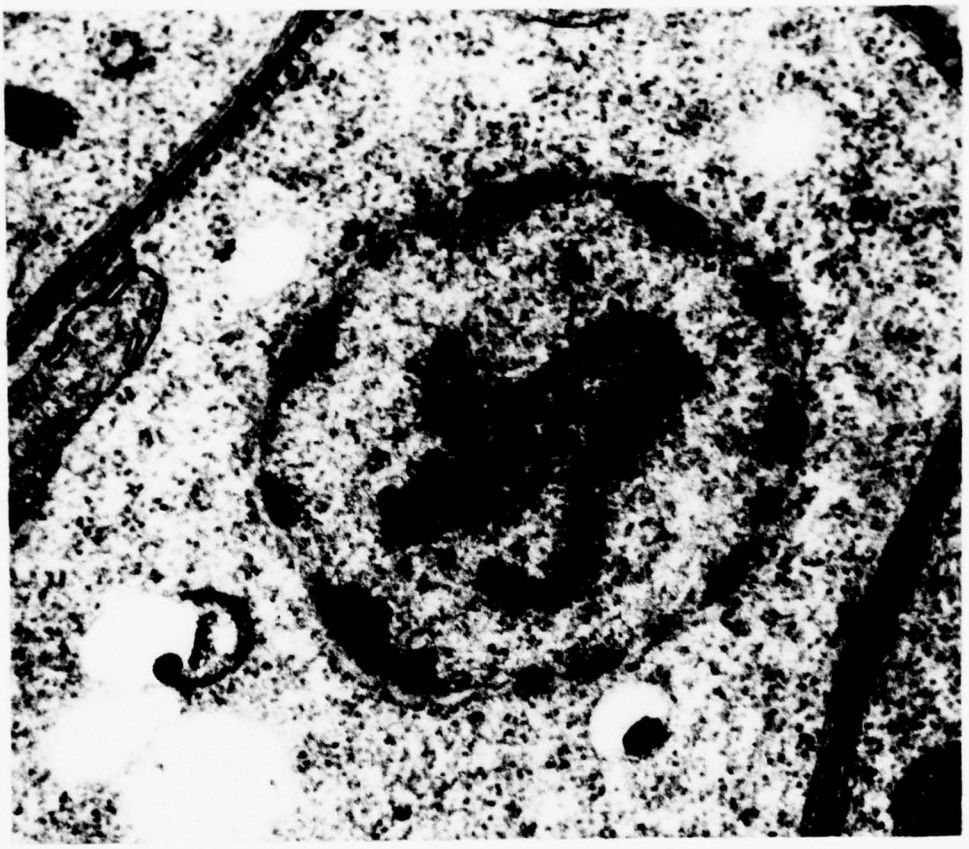

FIGURE 2

L. hertigi showing paracrystalline array of ribosome-like particles.

x 60,000

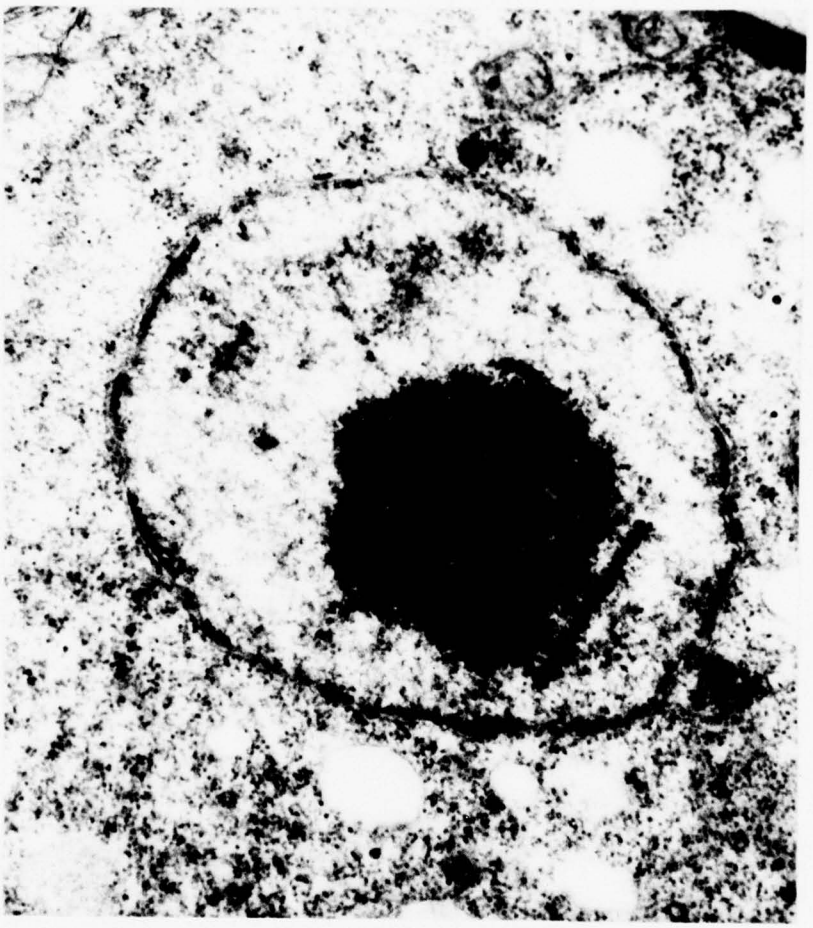

FIGURE 3

L. hertigi showing helical arrangement of ribosome-like particles.

x 60,000

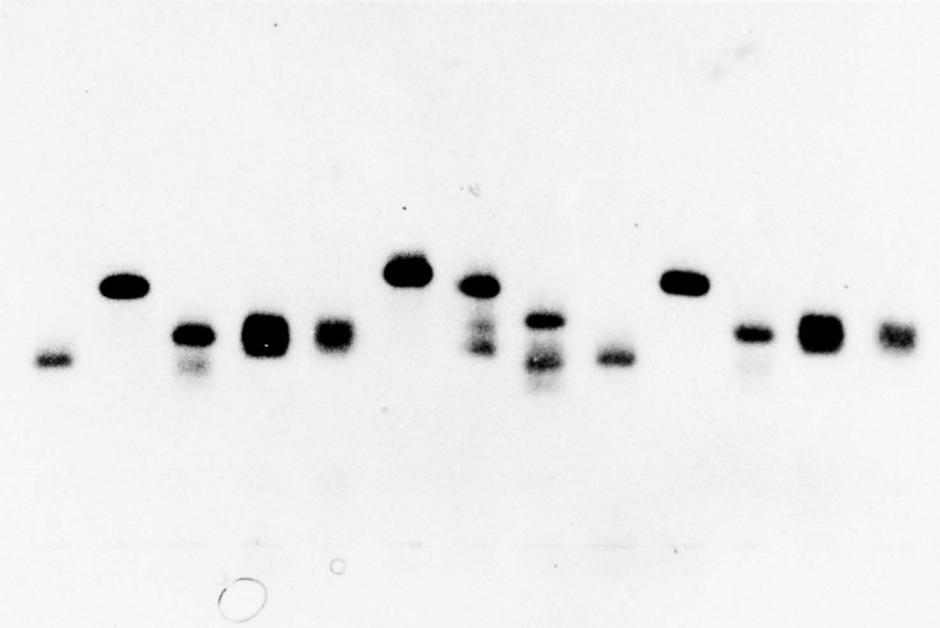

FIGURE 4

Enzyme variants of glucose phosphate isomerase.

Order of strains left to right: 1) LV29 *L. aethiopica*
2) LV9 *L. donovani* Ethiopia 3) LV487 *L. sp.* Namibia *P. rossi*
4) LV425 Namibia human 5) LV386 *L. sp.* Namibia human
6) LV388 *L. sp.* Arvicanthis Ethiopia 7) LV114 "*L. infantum*"
France 8) LV39 *L. major* 9) LV29 10) LV9 11) LV487
12) LV425 13) LV386

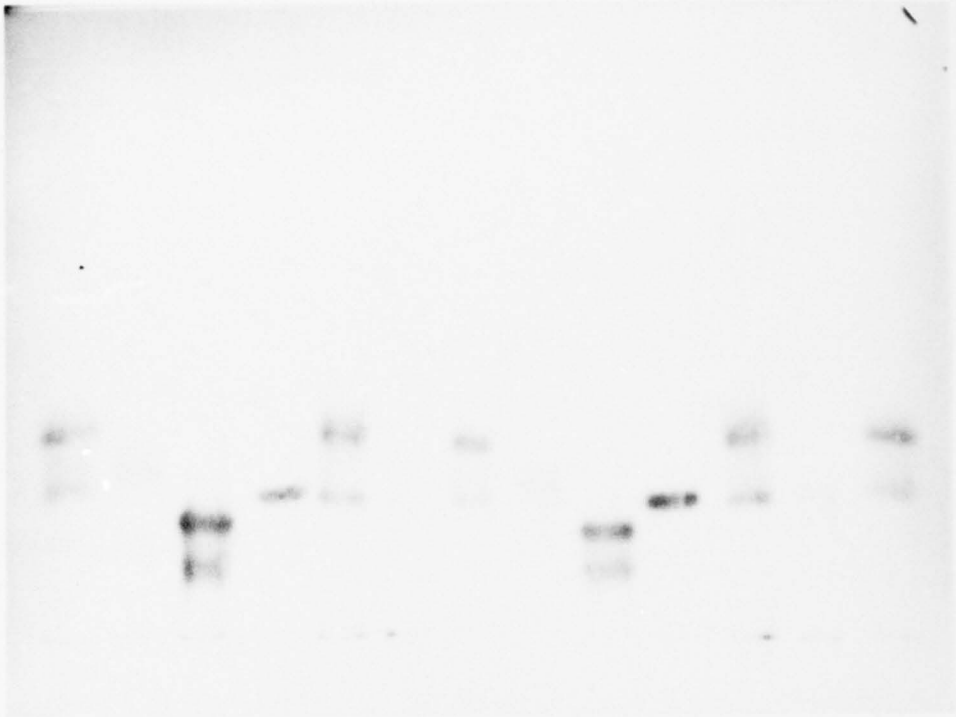

FIGURE 5

Enzyme variants of glucose phosphate isomerase.

Order of strains left to right: 1) LV9 L. donovani Ethiopia
2) LV425 L. sp. Namibia human 3) LV489 L. sp. Namibia P. rossi
4) LV181 L. sp. Tatera Kenya 5) LV114 "L. infantum" France
6) LV405 L. sp. France human O.S. 7) LV9 8) LV425
9) LV489 10) LV181 11) LV114 12) LV405 13) LV9

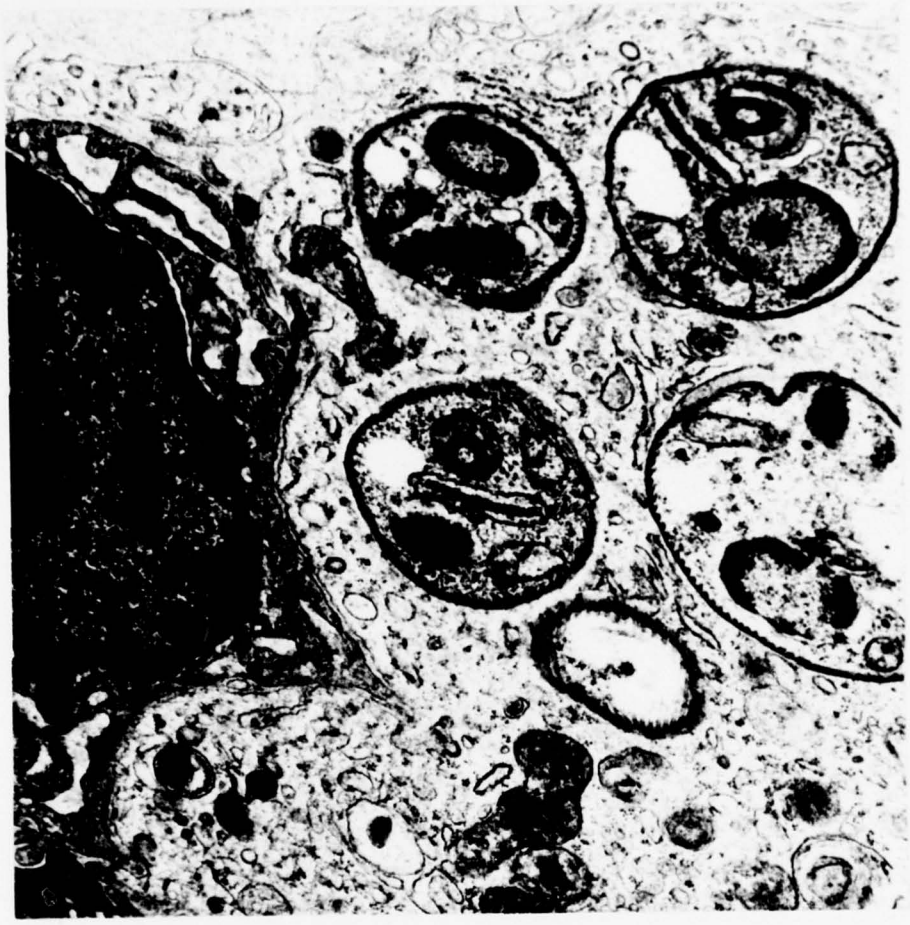

FIGURE 6

Effect of a single subcutaneous dose of Pentostam (400 mg base/kg) on "L. donovani s. l." LV9 infection in mouse liver showing ribosome diminution.

x 30,000

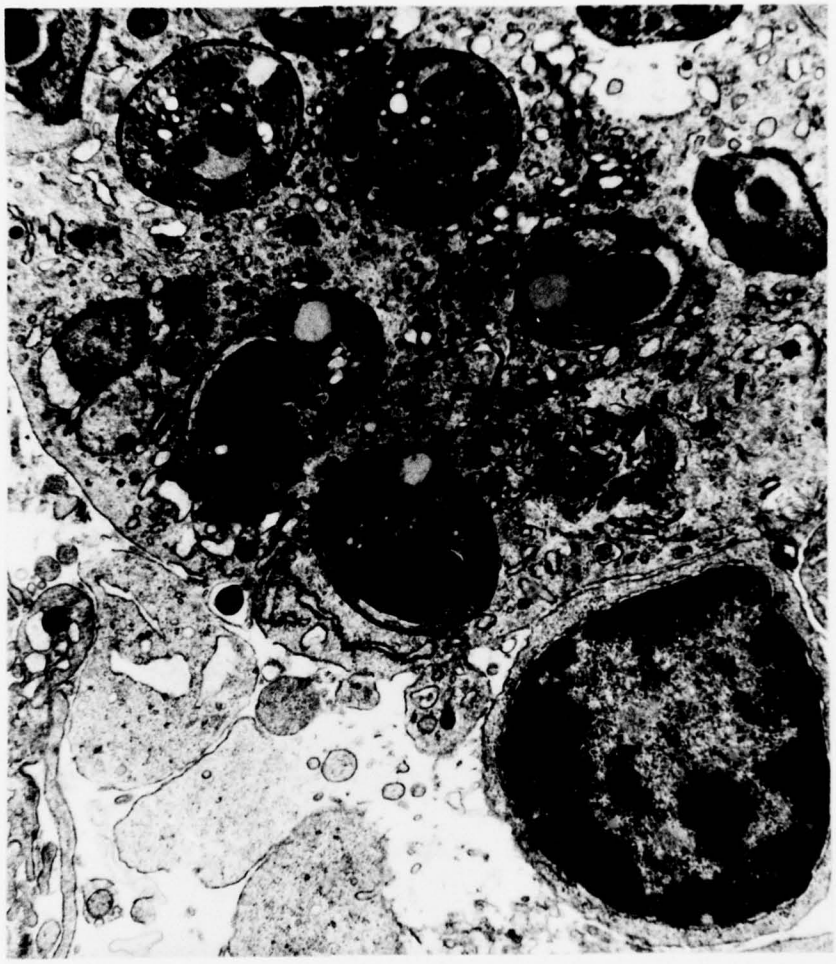

FIGURE 7

Untreated "L. donovani s. l." LV9 infection in mouse liver

x 40,000

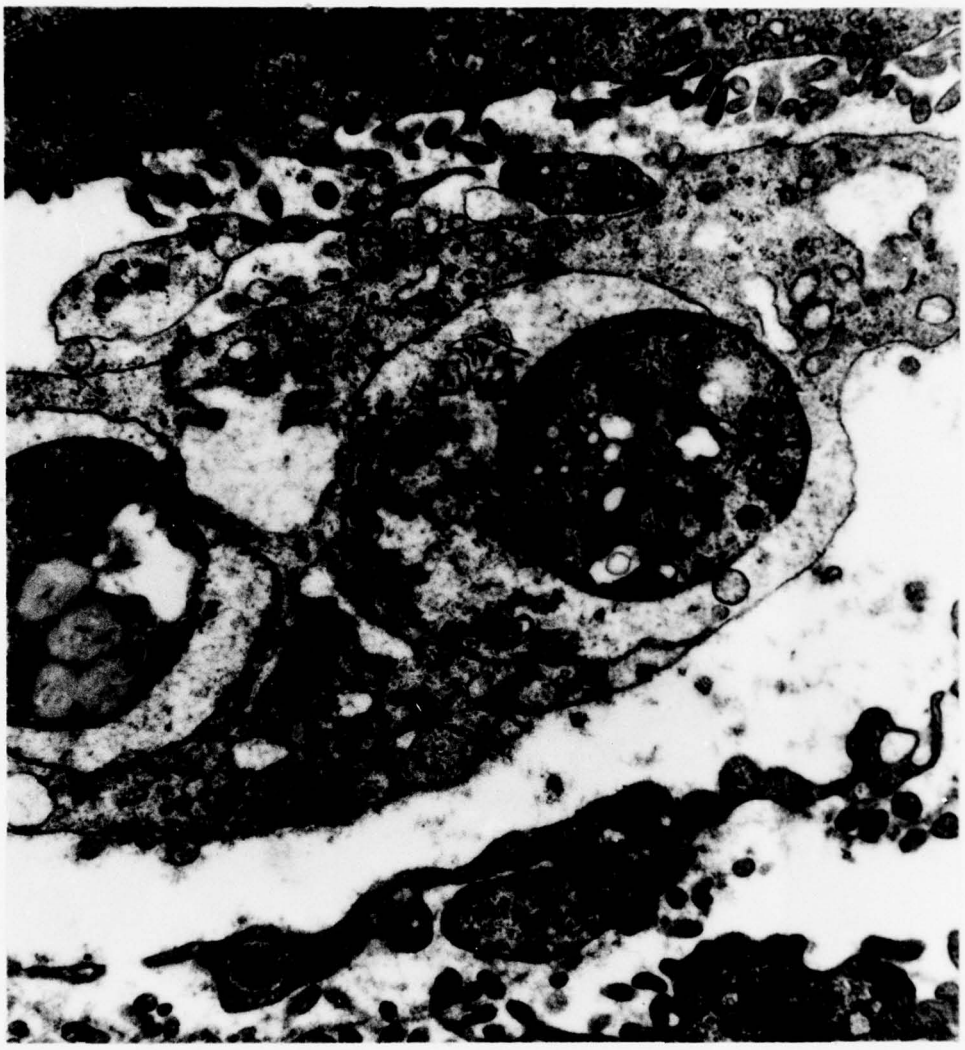

FIGURE 8

Untreated "L. donovani s. l." LV9 infection in mouse liver showing debris of membranous structure within partly formed parasitophorous vacuole.

x 20,000

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The resistance of intracellular *Leishmania* parasites to digestion by lysosomal enzymes

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Organisms of the genus *Leishmania* are obligate intracellular parasites of vertebrate mononuclear phagocytes. They share with such organisms as *Toxoplasma gondii*, *Trypanosoma cruzi* and certain species of mycobacteria, the ability to survive within a type of cell noted for its microbicidal properties (Cohn, 1963). In most cases the method of survival involves avoiding contact with host-cell lysosomal enzymes. However, the means by which this is achieved varies according to the species of parasite. *Toxoplasma gondii* can inhibit lysosomal fusion with the parasitophorous vacuole (Jones and Hirsch, 1972), although whether this constitutes the sole means of protection is uncertain. *Mycobacterium tuberculosis* can also inhibit lysosomal fusion (Armstrong and Hart, 1971), although *M. lepraemurium* achieves immunity through a physical barrier in the form of a thick enveloping capsule (Draper and Rees, 1970, 1973). *Trypanosoma cruzi* is able to escape from the original phagosome and live in direct contact with the cytoplasm (Peters, 1974; Kress *et al.*, 1975) so avoiding the essentially vacuole-bound lysosomal enzymes. Studies of *Leishmania* in macrophages (Alexander, 1974; Lewis, 1975; Alexander and Vickerman, 1975) indicated that lysosomal fusion with parasitophorous vacuoles takes place as normal. In the present study we consider several aspects of the relationship between *Leishmania* parasites and host macrophages, and their relevance to the intracellular survival of the protozoan.

MATERIALS AND METHODS

Parasite Strains

Promastigote forms of *Leishmania mexicana mexicana* (Liverpool code number LV4), *L. m. amazonensis* (LV79), *L. braziliensis braziliensis* (LV64) and *L. enriettii* (LV90) were cultured routinely in Locke's solution over human or rabbit blood-agar NNN slopes. The history of these strains was described by Chance *et al.* (1974) and Gardener (1975). *L. b. braziliensis* was adapted to grow in biphasic culture by Hommel *et al.* (1975). Cultures of all these organisms had lost the ability to produce lesions in hamsters or, in the case of *L. enriettii*, guinea pigs.

Amastigote forms of *L. m. mexicana* and *L. m. amazonensis* were obtained from cutaneous lesions in the foot-pads of hamsters.

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Isolation and Maintenance of Macrophages

Peritoneal exudates were collected from TFW outbred, or C57 black inbred mice, by standard procedures. Donor animals used as sources of normal macrophages were either untreated, or had been inoculated intraperitoneally with 3 ml of 2% rice starch before isolating the cells ('induced' macrophages). Cells were cultured in Leighton tubes or 500 ml tissue-culture flasks (for electron microscopy), using tissue-culture medium 199 with 20% foetal bovine serum, buffered with HEPES. Non glass-adherent peritoneal exudate factors were removed by rinsing before introducing parasites if the experimental design so demanded.

Activation of Macrophages

Macrophages were activated either *in vivo* before isolation, or *in vitro*, after the cultures were established. For activation *in vivo*, donor mice were inoculated intraperitoneally with 0·5 ml of emulsion made from equal proportions of Freund's complete adjuvant and a lysate of *L. m. mexicana* promastigotes, repeated after seven days. Macrophages were harvested three days after the second inoculation, using heparin in the lavage medium to reduce clumping. The suspension of cells so obtained was centrifuged at 8000 g for five minutes in ice-cold centrifuge tubes so that the oily adjuvant residues could be aspirated from the top of the supernatant. Cells were resuspended in fresh medium and dispensed into the culture vessels.

Macrophages were activated *in vitro* by lymphocyte-derived factors (lymphokines: Dumonde *et al.*, 1969) produced by a technique derived from those of Nath *et al.* (1973) and Mauel and Behin (in preparation). Macrophages were collected from mice given 0.3 ml Freund's complete adjuvant intraperitoneally six days previously. The spleens were removed aseptically from the same mice, homogenized in culture medium, and the homogenate stored at 4°C until ready for use. The macrophages were cultured as normal, and 12 hours after introducing the parasites, syngeneic lymphocytes were added in the proportion of three to five per macrophage. The plant mitogen Concanavalin A was then added to give a final concentration of 7 μg/ml. In controls either the lymphocytes or the Concanavalin A were omitted, and both types of control were incorporated with each experiment.

Both techniques induced behavioural and morphological changes commensurate in all respects with the process of activation described by Mackaness (1970), Nathan, Karnovsky and David (1971), Nath, Poulter and Turk (1973) and Nathan, Remold and David (1973).

Infection of Macrophages

Leishmania organisms, in either the promastigote or amastigote form, were added to the tissue cultures in the proportion three to five parasites per macrophage. In most cases subsequent incubation was at 31–32°C but cultures containing *L. m. amazonensis* and *L. b. braziliensis* were also incubated at 36–37°C.

Preparation for the Light Microscope

Cover slips were fixed *in situ* with Zenker's solution plus 5% acetic acid, and stained with 5% Giemsa stain. The progress of infection in the macrophage populations was assessed by examining 24 or 48 hour samples, taking three replicates per treatment. The levels of infection were quantified in terms of the proportion and actual number of cells infected, together with the mean numbers of parasites per infected cell. This was derived from studies of 20 fields ($\times$1000 magnification) randomly distributed across the length of each cover slip.

Preparation for the Electron Microscope

Macrophages were cultured in 500 ml 'Flow' tissue culture bottles, and fixed *in situ* by a

method similar to that of Hirsch and Fedorko (1968). Fixed cells were scraped off the surface of the glass vessel using a 'rubber policeman' and enmeshed in a fibrin clot to facilitate handling (Lewis, 1974). Fragments of these clots were dehydrated in an ethanol series, embedded in TAAB epoxy resin and sectioned in an LKB ultratome MKIII. Sections were stained in uranyl acetate and (except for enzyme histochemistry) lead citrate, and examined in an AEI EM6B electron microscope.

Labelling of Secondary Lysosomes

This technique was principally that described by Armstrong and Hart (1971) and subsequently applied to protozoan intracellular parasites by Jones *et al.* (1972). Two different labels were used in this study. (a) Colloidal thorium hydroxide: available from TAAB Laboratories Ltd., this was prepared as a 33% w/v stock colloidal suspension in 1% acetic acid. Cultures containing 100 ml of medium were treated with 0·2 ml of the stock suspension and incubated at 36–37°C for four hours. (b) Saccharated iron oxide: also available from TAAB Laboratories Ltd., already in colloidal suspension. Cultures were incubated with the colloid at a concentration of 3% v/v, for five hours at 36–37°C.

At the end of the labelling period 'monolayers' were thoroughly rinsed, the medium renewed, and the cultures were left for two to three hours at 36–37°C to allow complete internalization of the electron dense particles. *Leishmania* organisms were then added in the usual way.

Histochemical Localization of Acid Phosphatase

Infected macrophages were prepared for electron microscopical lozalization of acid phosphatase activity by the method of Cohn *et al.* (1966). However the formulation of the incubation medium was according to the revised Gomori technique, after Barka and Anderson (1962).

RESULTS

Infection of Normal Mouse Peritoneal Macrophages by *Leishmania* Amastigotes

When amastigote forms of *L. m. mexicana* or *L. m. amazonensis* were isolated from a hamster cutaneous lesion and used to infect cultures of induced mouse peritoneal macrophages, most of the parasites were phagocytosed within 12 to 24 hours. The majority of the intracellular parasites were already surrounded by a recognizable 'halo' representing the interior of the phagosome/parasitophorous vacuole. This vacuole enlarged considerably over the ensuing 24–48 hours (Fig. 1) and the intracellular parasites multiplied. After a time (which varied according to the condition of the cultures, generally within six days) most macrophages appeared packed with parasites. Many cells were caused to lyse, releasing their parasites which then reinvaded other cells. Therefore from this point onwards, the number of cells attached to the cover slips often decreased.

Infection of Normal Mouse Peritoneal Macrophages by *Leishmania* Promastigotes from Culture

In contrast to experiments using amastigote inocula, results with promastigote inocula differed according to the species of *Leishmania*. In all cases, however, the proportion of the inoculum that infected macrophages was smaller than when amastigotes were used, and infections developed more slowly.

After invasion of the macrophage, the behaviour of the parasites appeared similar to that described by Akiyama and Haight (1971). Thus there was evidence that a considerable proportion of the recently endocytosed promastigotes was damaged. This feature was

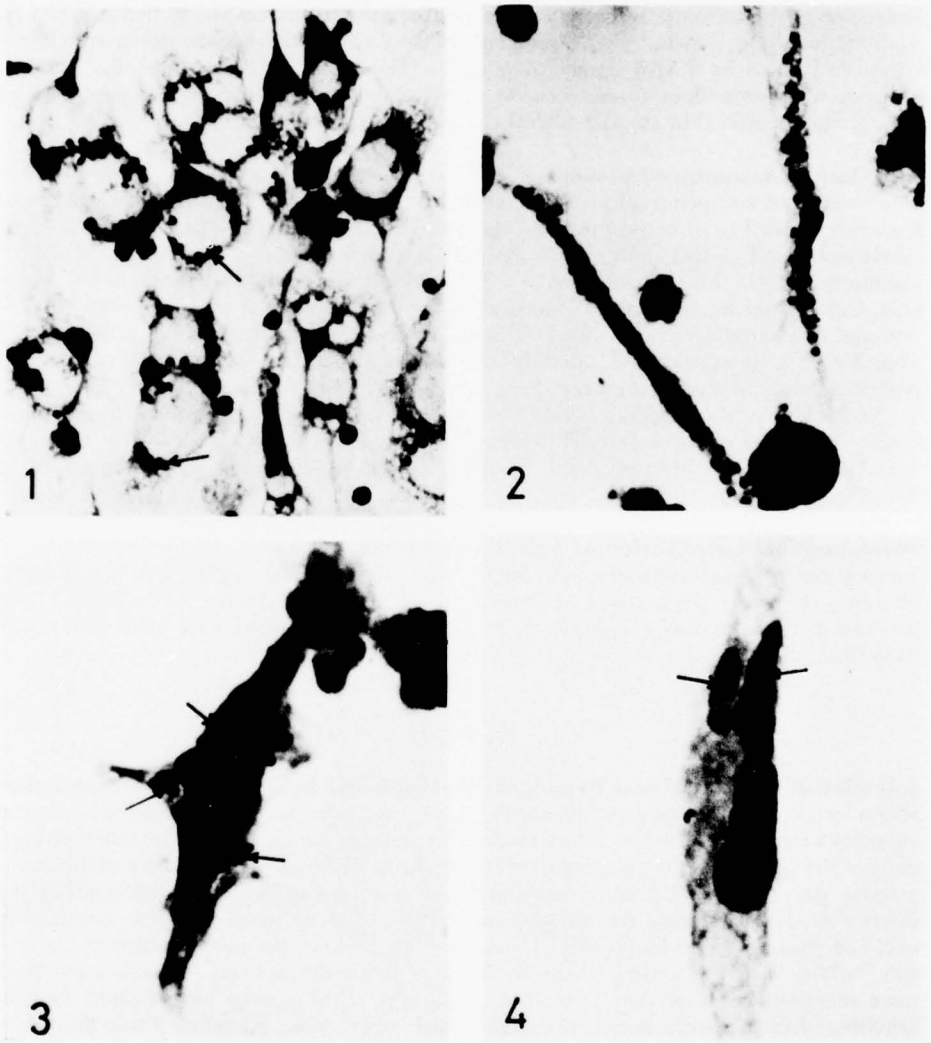


Fig. 1. Light micrograph of induced mouse peritoneal macrophages infected with lesion-derived amastigote forms of *L. m. mexicana*. The preparation was fixed four days after introducing the parasites. Parasites (arrowed) are situated within greatly distended vacuoles (× 720).

Fig. 2. Light micrograph of induced mouse peritoneal macrophages also containing four-day-old infections of *L. m. mexicana*, but this time derived from cultured promastigote forms. Note the absence of large vacuoles and the more normal spindle-shape of the cell (× 900).

Fig. 3. Light micrograph of an induced mouse peritoneal macrophage after 24 hours' association with promastigote forms of *L. m. mexicana*. Most of the intracellular parasites have rounded up and become amastigote-like (arrowed) (× 1800).

Fig. 4. Light micrograph of an induced mouse peritoneal macrophage after 24 hours' association with promastigote forms of *L. m. amazonensis*. Note the more elongate nature of the intracellular parasites (arrowed) indicating the incomplete transformation typical of cultured forms of this species (× 1800).

more pronounced in cultures inoculated with *L. m. amazonensis* than in those inoculated with *L. m. mexicana*. Also, early infections of *L. m. amazonensis* showed parasites of a more elongate shape than *L. m. mexicana* infections of similar age (Figs. 3 and 4), suggesting that transformation was incomplete. In neither case was there visible (with the light microscope) evidence of pronounced vacuole development around individual parasites. Clear 'haloes' became significant only amongst two to three day old infections, and they never reached anything approaching the size of the vacuoles associated with infections of lesion-derived amastigotes (Figs. 1 and 2).

After 24 to 48 hours, infections of *L. m. mexicana* developed differently from those of *L. m. amazonensis*. With *L. m. mexicana* the proportion of apparently damaged intracellular parasites declined, and the numbers of what appeared to be intracellular amastigotes gradually increased. It was difficult to ascertain what proportion of this increase was due to multiplication of intracellular parasites, and what was due to continued invasion by free promastigotes. Even thorough rinsing failed to remove extracellular promastigotes that were attached to cells. Dividing forms, in the shape of stumpy-to-rounded promastigotes, were seen both inside and outside cells (Fig. 11). From day 3 onwards the cultures followed a similar course to those inoculated with lesion-derived amastigotes.

Infections of *L. m. amazonensis* did not usually increase, and the proportion of damaged intracellular parasites remained similar to that which was evident during the first 24 hours. Whilst extracellular parasites divided and maintained full motility at 37°C, intracellular parasites appeared not to survive. Reducing the temperature of incubation to 32°C (that used for *L. m. mexicana* cultures) made no difference to this situation. Cultured promastigote forms of *L. b. braziliensis* and *L. enriettii* behaved in a similar manner to *L. m. amazonensis* after ingestion by normal macrophages. These species also retained an elongate configuration within the macrophages, suggesting either that they failed to transform, or that they were digested before transformation could occur.

TABLE 1

Mean numbers of L. m. mexicana *amastigotes per infected macrophage, in cultures of normal and activated macrophages. Cultures were washed before infection (data from the experiment also represented by Fig. 5)*

Culture	*Time of association*			
	One day	*Two days*	*Three days*	*Four days*
Normal macrophages	3·2	4·9	4·4	1·5
	2·9	4·6	2·9	5·8
	2·9	5·0	5·4	6·1
	$\bar{x}$ 3·0	$\bar{x}$ 4·8	$\bar{x}$ 4·2	$\bar{x}$ 4·4
Activated macrophages	2·0	3·4	3·5	4·3
	2·0	2·8	4·1	4·4
	2·1	3·2	—	3·9
	$\bar{x}$ 2·0	$\bar{x}$ 3·1	$\bar{x}$ 3·8	$\bar{x}$ 4·2

Effect of Macrophage Activation on *Leishmania* infection

The effects of macrophage activation on intracellular *Leishmania* varied according to the method and time of activation and also the morphological form of the infecting parasites. Fig. 5 illustrates a typical result obtained when populations were activated *in vivo*, and the subsequent cultures rinsed prior to infection with promastigote forms of *L. m. mexicana*. After 24 hours' association the level of infection of activated macrophage populations was

significantly less than that of normal macrophage populations, both in terms of the proportion/total number of cells infected, and also the mean number of amastigotes per infected cell (Table 1). However, subsequent samples showed a progressive reduction in this difference such that, after two to three days, infections of normal and activated macrophage populations were similar. The difference between infections of control and activated populations was less when the former were represented by induced macrophage populations than when non-induced cells were used (Fig. 6). If activated macrophage

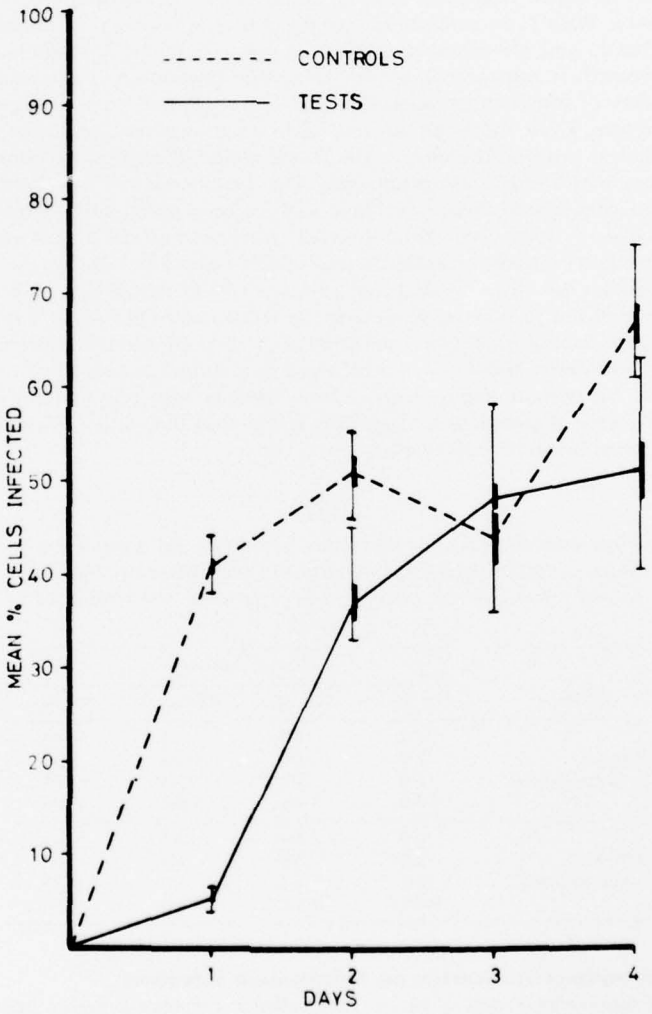


Fig. 5. Graph describing the levels of infection in populations of normal (controls) and activated (tests) macrophages associated with promastigote forms of *L. m. mexicana* over a period of four days. Each point represents the mean value from three replicates. The thin vertical bars indicate the range, and the thick vertical bars represent the standard error.

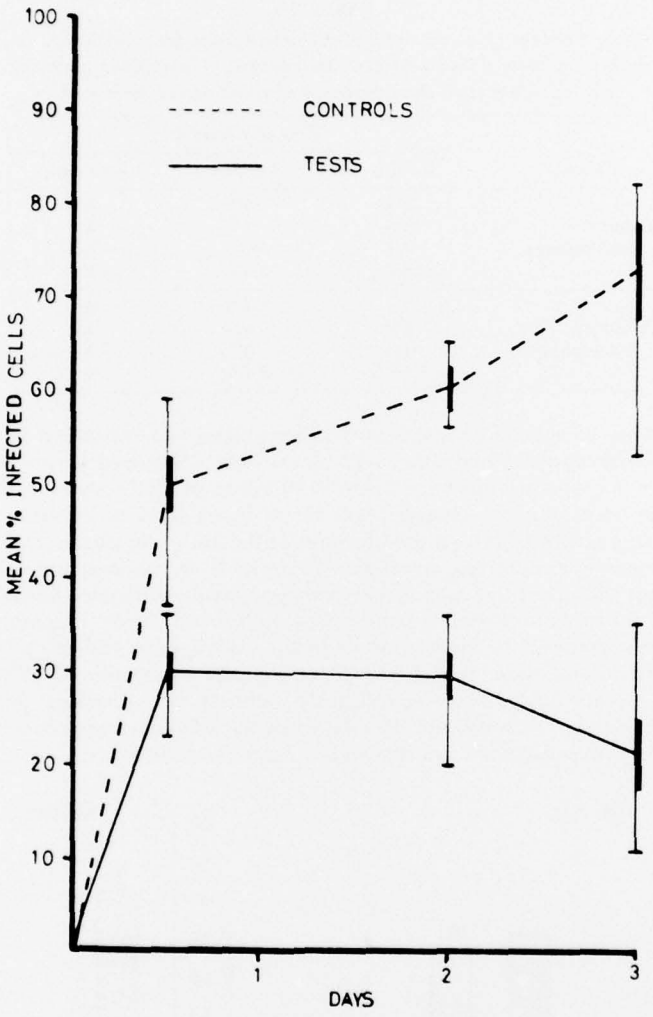


Fig. 6. Graph describing the results of an experiment similar to that represented by Fig. 5. However in this case control macrophages were induced, and the macrophage 'monolayers' were not washed after introducing the peritoneal exudate into the culture tubes.

'monolayers' were not rinsed before exposure to *L. m. mexicana* promastigotes, then the decreased level of infection persisted for several more days (Fig. 6 and Table 2).

It was possible that the lower level of infection in cultures of activated macrophages was caused either by extracellular immunoglobulins, or by differences in the phagocytic ability of normal and activated macrophages. To test the former, promastigote forms of *L. m. mexicana* were incubated in cell-free exudate from sensitized mice for five days. During this time the *Leishmania* showed no significant loss of motility, inhibition of division, or agglutination compared with control populations in exudate from normal mice. The

TABLE 2

Mean numbers of L. m. mexicana *amastigotes per infected cell in cultures of induced and activated macrophages. The cultures were not washed (data from the experiment also represented by Fig. 6)*

Culture	*Time of association*		
	One day	*Two days*	*Three days*
Induced macrophages	3·4	5·5	5·2
	3·6	4·7	9·0
	2·7	—	8·8
	$\bar{x}$ 3·2	$\bar{x}$ 5·1	$\bar{x}$ 7·6
Activated macrophages	3·7	2·9	4·5
	2·9	4·1	4·7
	2·5	3·5	5·4
	$\bar{x}$ 3·0	$\bar{x}$ 3·5	$\bar{x}$ 4·8

phagocytic ability of normal and activated macrophages was compared in terms of the number of fixed sheep red blood cells endocytosed over a standard period of association. Both types of macrophage endocytosed similar numbers of erythrocytes during 24 hours, and there was no evidence to suggest that the reduced level of infection in activated macrophages was attributable to a modification of the ability to phagocytose.

When hamster lesion-derived amastigote forms of *L. m. mexicana* were used to infect the macrophage cultures there was rarely any appreciable difference between the levels of infection of activated and control populations. Fig. 7 and Table 3 represent the results of an experiment designed to compare the effect of macrophage activation when either a promastigote or an amastigote inoculum of *L. m. mexicana* was employed. Whilst infections produced by promastigotes were significantly reduced in unwashed preparations of activated macrophages, such was not the case when the infection was produced by amastigote forms. These experiments were repeated at least three times.

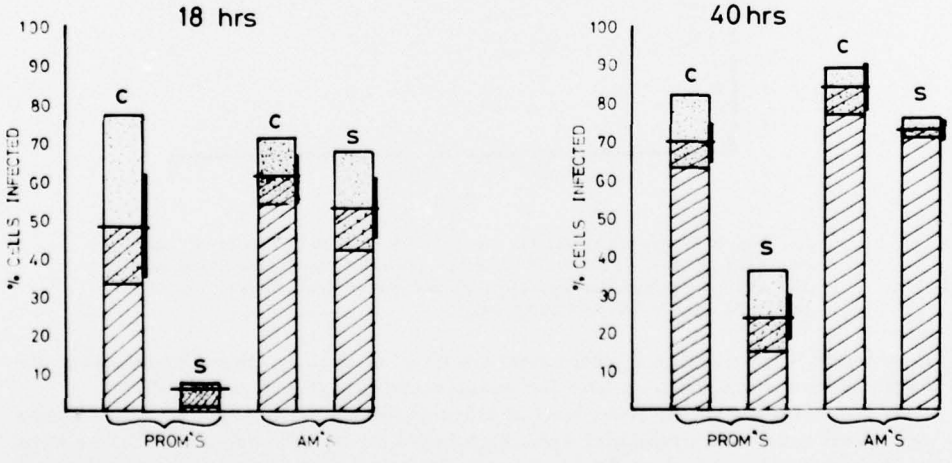


Fig. 7. Histograms depicting the comparative effects of macrophage activation on infections derived from promastigote and amastigote forms of *L. m. mexicana*. C—control (normal) macrophages; S—activated macrophages; Am's—amastigote inocula; Prom's—promastigote inocula. Stippled areas denote range and thick vertical bars represent the standard error.

TABLE 3

Mean numbers of amastigotes per infected macrophage. Data taken from an experiment to compare the effects of activation on infections derived from amastigote and promastigote forms of L. m. mexicana (*data from the experiment also represented by Fig. 7*)

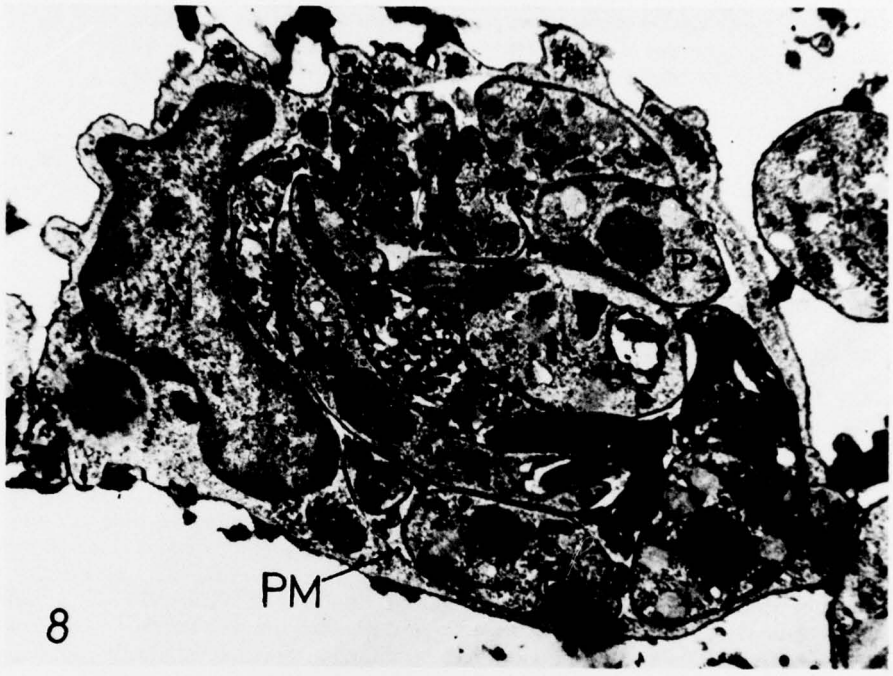
Time of association	*Culture*			
	Normal macrophages infected with promastigotes	*Activated macrophages infected with promastigotes*	*Normal macrophages infected with amastigotes*	*Activated macrophages infected with amastigotes*
18 hours	4·0	5·0	3·4	3·5
	5·9	3·0	4·1	2·9
	3·8	2·6	4·3	3·6
	$\bar{x}$ 4·5	$\bar{x}$ 3·5	$\bar{x}$ 3·9	$\bar{x}$ 3·3
40 hours	5·4	3·9	5·1	4·2
	5·7	1·7	5·6	4·1
	7·2	3·6	5·2	—
	$\bar{x}$ 6·1	$\bar{x}$ 3·0	$\bar{x}$ 5·3	$\bar{x}$ 4·1

When macrophage monolayers were activated *in vitro* after infection, different results were obtained. In such cases activation of macrophage populations had no recognizable effect on the level of infection compared with populations of normal cells, irrespective of the form in which the parasite was first introduced. By this method the cells only attained an appreciable level of activation at least three days after the first parasites were endocytosed. During this period, the majority of promastigotes completed transformation to the amastigote form.

Activation *in vitro* produced no recognizable change in the levels of infection achieved by those species of *Leishmania* promastigote that survived poorly in normal macrophages. Thus infections derived from promastigote forms of *L. enriettii*, *L. b. braziliensis* and *L. m. amazonensis* remained small, with a high proportion of damaged parasites in both activated and normal cells. It is, however, possible that an enhanced lytic ability in activated cells might have been balanced by proportionately enhanced phagocytosis of free[illegible]es.

Ultrastructural Observations

Preparations of normal macrophage cultures fixed 48 hours after introducing *L. m. mexicana* promastigotes showed intracellular parasites in varying stages of transformation and degeneration when viewed with the electron microscope. Many of these different stages were often represented within single cells (Fig. 8). All the intracellular parasites were surrounded by a membrane, presumably derived from the macrophage cell membrane, which defined the parasitophorous vacuole (Figs. 9, 10, 11). From consideration of the stage in the transformation of the parasite and the time elapsed between inoculation and fixation, it seemed that recently phagocytosed parasites were closely surrounded by the phagosomal membrane (Fig. 9). Older infections typically showed distension of the parasitophorous vacuole so that the phagosomal membrane was widely separated from the parasite for most of its area (Figs. 12, 13). This distension was caused by accumulation of fluid around the parasite, and the source of the fluid will be discussed. There was no apparent correlation between the size of the parasitophorous vacuole and the viability of the contained parasite. Many of the intracellular *Leishmania* showed morphological evidence of active secretion from their flagellar pockets, in the form of small vesicles which corresponded with similar vesicles found free in the body of the vacuole (Fig. 10).


PM
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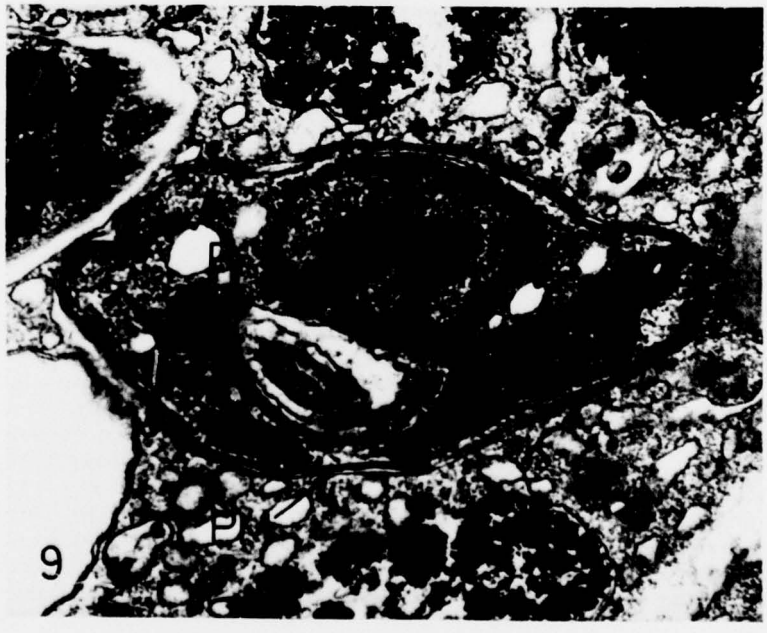
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Similar preparations of cultures inoculated with amastigote forms mainly revealed parasitophorous vacuoles of the more distended type, with less evidence of a pre-distension stage. At no time was any evidence obtained (under the conditions of fixation and staining that we employed) of parasites in direct contact with host cytoplasm, or of any surface coat surrounding the parasites that might serve as a physical barrier to digestive enzymes.

Lysosomal Interactions

Both types of colloidal label were readily endocytosed by macrophages, and could subsequently be demonstrated within vacuoles of a wide size range. However the distribution of the labels relative to intracellular *Leishmania* varied according to type. In normal macrophages labelled with saccharated iron oxide, and infected with either amastigote or promastigote forms of *L. m. mexicana*, the majority of parasitophorous vacuoles contained colloidal particles and were closely surrounded by labelled secondary lysosomes. Some of these were often fixed during the actual process of fusion with the parasitophorous vacuole (Fig. 11). Preparations of similar cultures labelled with thorium hydroxide also showed parasitophorous vacuoles surrounded by labelled secondary lysosomes (Fig. 9). Many of these parasitophorous vacuoles themselves contained label (Fig. 12), but this was seen less frequently than in cells labelled with saccharated iron oxide.

Irrespective of the type of label, there was little correlation between the presence of colloidal particles within a parasitophorous vacuole, and the apparent viability of the enclosed parasite. It appeared that fusion of secondary lysosomes with parasitophorous vacuoles was indiscriminate. In view of the fact that an ultrathin section only samples a small portion of the parasitophorous vacuole the incidence of labelling was high enough to assume that all such vacuoles coalesce with secondary lysosomes.

Acid Phosphatase Activity

Acid phosphatase activity within the parasitophorous vacuoles of normal macrophages infected with promastigote forms of *L. m. mexicana*, appeared to be low. Lead phosphate deposition was confined to the periphery of the vacuoles, even though the parasites themselves showed localizations of acid phosphatase activity (Fig. 13) particularly in the flagellar pocket. Thus it was evident that the reaction mixture was penetrating the vacuole adequately. Autosomal vacuoles in non-infected cells, on the other hand, showed dense lead deposition distributed throughout their interiors (Fig. 14), signifying that the distribution typical of parasite-containing phagosomes was not artefact. The amount of lead deposit did not increase with the volume of the parasitophorous vacuole, and was maximal before fluid distension occurred.

Effects of Macrophage Activation on Lysosomal Activity

Electron microscope observation of activated macrophages labelled with electron dense particles revealed only quantitative changes in the lysosomal response to intracellular *L. m. mexicana* organisms. It proved difficult to characterize such changes statistically due to

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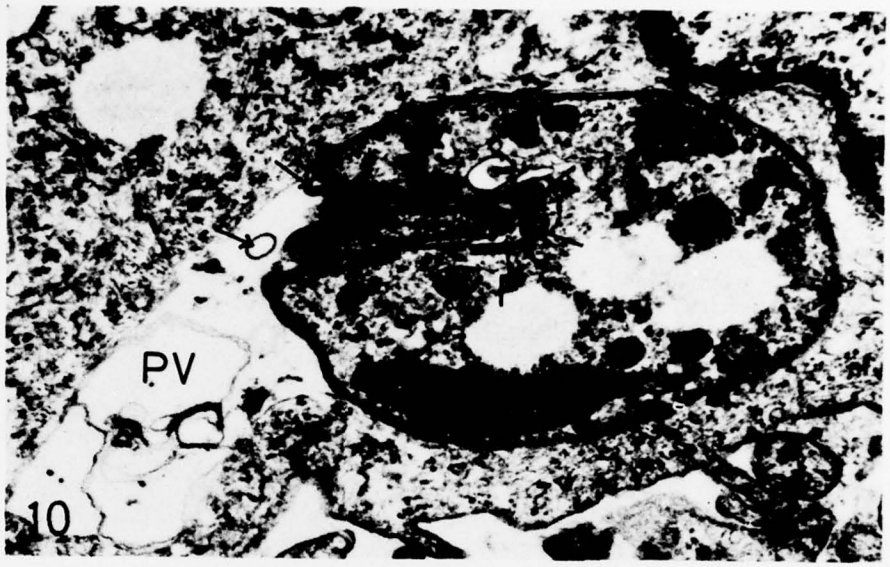
Fig. 8. Electron micrograph of an induced mouse peritoneal macrophage after 24 hours' association with *L. m. mexicana* promastigote forms. A single parasitophorous vacuole, defined by the phagosomal membrane (PM) contains *Leishmania* parasites (P) in various stages of transformation. The macrophage nucleus (N) lies to one side (×8000).

Fig. 9. Electron micrograph of a recently endocytosed *Leishmania* which has nearly completed transformation to the amastigote form. The phagosomal membrane (PM) closely surrounds the parasite (P). Secondary lysosomes containing particles of thorium hydroxide surround the parasitophorous vacuole. Other features include the kinetoplast (K), nucleus (N) and flagellar pocket (×30 000).



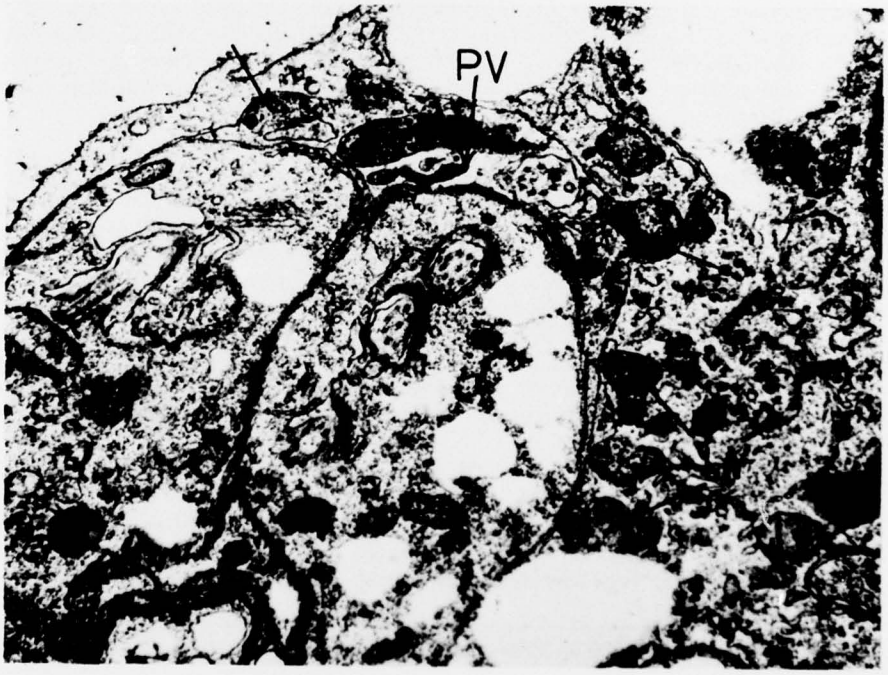


Fig. 10. Electron micrograph showing an intracellular *L. m. mexicana* parasite. Vesicles (arrowed) free in the parasitophorous vacuole (PV) probably originate from the flagellar pocket (F). Similar vesicles are visible at the base of the flagellar pocket and in the cytoplasm of the parasite (arrowed) (×20·000).

Fig. 11. Electron micrograph of a macrophage labelled with saccharated iron oxide and then infected with *L. m. mexicana* promastigote forms. Labelled secondary lysosomes (LY) surround the parasitophorous vacuole. A lysosome has been fixed in the process of fusing with the vacuole (arrowed). Particles of label surround the parasites, one of which is dividing and has two flagellar axonemes (×20·000).

the great variance in the amount of label taken up by individual cells. Activated macrophages took up more label than normal macrophages and a larger proportion of parasitophorous vacuoles contained the label. Surprisingly the incidence of damaged parasites appeared similar in activated and normal macrophage populations.

DISCUSSION

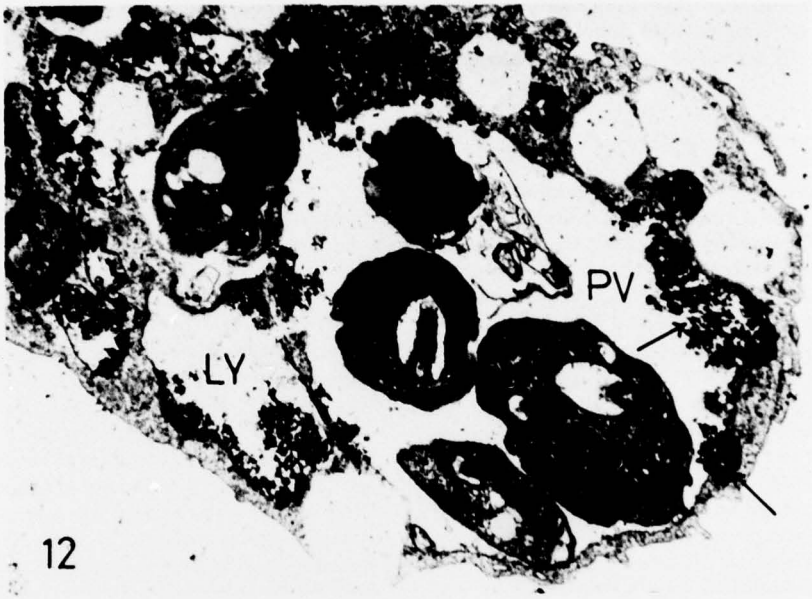
Leishmania in Normal Macrophages

There was a considerable difference between macrophage infections derived from endocytosed promastigotes and those from endocytosed amastigotes. This was particularly evident with regard to the size of the parasitophorous vacuole, which enlarged very rapidly around amastigote forms, but took several days to enlarge progressively around intracellular parasites derived from promastigote 'invaders'. Therefore under the conditions of the present study the size of the parasitophorous vacuole probably reflected the stage in transformation of the parasite towards the amastigote form. Akiyama and Haight (1971) and Akiyama and McQuillen (1972) observed that only moribund forms of *L. donovani* were associated with large parasitophorous vacuoles. In our experience the opposite was true, and only the most viable infections were associated with large vacuoles both *in vitro* and *in vivo* (unpublished observations). This discrepancy may be related to the observations of Frothingham and Lehtimaki (1967) and Gardener (1974) who describe distinct species differences in the size of the parasitophorous vacuole. Our own observations indicate that the main factor affecting survival of endocytosed promastigotes is their ability to transform. Thus lesion-derived amastigote forms of *L. m. amazonensis* survived well within normal macrophages, while promastigotes from culture did not. In common with promastigotes of *L. enriettii* and *L. b. braziliensis*, *L. m. amazonensis* failed to transform with the same facility as *L. m. mexicana*. There is some evidence (Lewis, 1976) that the speed of transformation from promastigote to amastigote is closely related to the degree of virulence, as measured by the ability to produce lesions in hamsters. This view gains support from the concept of parasite 'competence', first outlined by Steinert (1958) and subsequently discussed by Deane and Kirchner (1963), and Trager (1968). All the cultured *Leishmania* species used in this study failed to produce lesions after subcutaneous inoculation into hamsters, but we have noticed that with time *L. m. mexicana* loses its virulence in culture far less rapidly than *L. m. amazonensis*.

Leishmania in 'Activated' Macrophages

The treatments used in this study, and the changes they produced, left little doubt that a significant measure of activation was achieved. However, we have so far been unable to incorporate controls of a type that would enable us to compare *the degree* of our 'activation' with that obtained by other workers. Only in this respect do we not completely satisfy the proposals directed by Allison and Davies (1975) towards descriptions of macrophage activation.

Macrophage activation produced a decrease in infection only when *L. m. mexicana* was introduced as promastigote forms after activation was complete. As the phagocytic ability of the cells appeared normal, and there was no evidence of any extracellular effects on the *Leishmania* parasites, this decrease must have been mediated intracellularly. Maintenance of this effect was dependent on additional non-macrophage factors present in the original peritoneal exudate, a feature also noticed by Bradley (personal communication cited by Mauel, Behin, Biroum-Noerjasin and Doyle, 1974) in a similar study. These factors were most probably lymphokines (Dumonde *et al.*, 1969) which characteristically act through the intermediacy of macrophages. The ability of lymphokine factors to maintain a decrease



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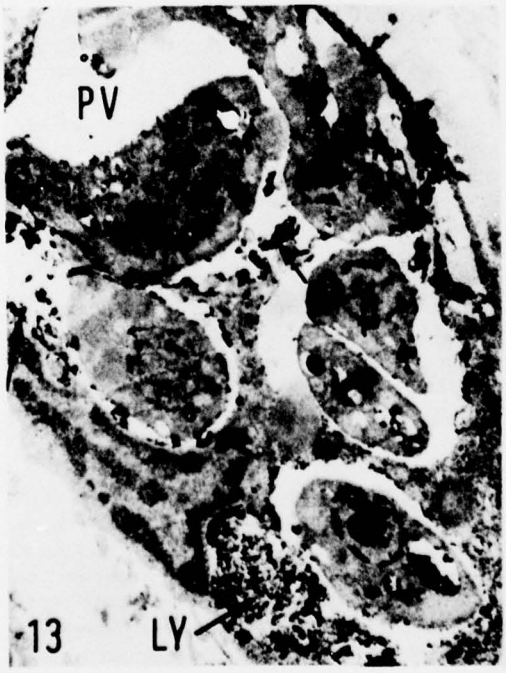

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in the level of *Leishmania* infections therefore supports the conclusion that the decrease was related to changes in the nature of the macrophages themselves.

If amastigote forms were used to infect activated macrophages, or if the macrophages were activated after promastigote forms had invaded and successfully transformed, then no reduction in the general level of infection was obtained. Thus, as in normal macrophages, it appears that only incompletely transformed promastigotes are vulnerable to the albeit enhanced degradative mechanisms of activated macrophages. Macrophage activation produces a decrease in infection by promastigote forms probably because the enhanced lysosomal response allows the parasite less time to transform. In this event the activated macrophage merely exploits more efficiently a vulnerability in the *Leishmania* that is equally evident in normal macrophages. Perhaps this is equally true *in vivo*; that is, does activation of the macrophage population only reduce the *Leishmania* infection if the parasite species is imperfectly adapted to survive in the normal macrophages of that species of host? The differences between normal and activated macrophages appear to be more quantitative than qualitative. Therefore if *Leishmania* parasites are able actively to counter the degradative capabilities of normal macrophages they should theoretically be able to do the same in activated cells. This general hypothesis may partly explain recent observations (Mauel *et al.*, 1975; Mauel and Behin, in preparation) leading to the conclusion that the survival of *Leishmania* in activated macrophages varies according to the species or strain of parasite and host animal. The hypothesis also carries the implication that if a *Leishmania* species is perfectly adapted to survive in the normal macrophages of a particular species of host, then activation of the macrophage population, on its own, will not be enough to achieve self-cure. Indeed there is evidence of this, as the onset of delayed hypersensitivity and lesion regression do not coincide in *L. tropica* infections of man (Adler 1964).

Our studies on the lysosomal response to intracellular *Leishmania* largely corroborate those of Alexander and Vickerman (1975) and Chang and Dwyer (1976). There is apparently no inhibition of lysosomal fusion such as occurs in macrophages infected with *T. gondii* (Jones and Hirsch, 1972). *Leishmania* parasites must therefore be able to resist the lysosomal enzymes themselves. This could be achieved either through a protective physical barrier as in the case of *Mycobacterium lepraemurium* (Draper and Rees, 1970; 1973) or through an active inhibition of the enzymes. Due to the lack of morphological evidence of a physical barrier, and the apparent low level of acid phosphatase activity within the parasitophorous vacuole, we consider the latter alternative the most likely. Such may be achieved through the agency of secreted products released from the flagellar pocket of the *Leishmania* parasite, an activity for which there was some ultrastructural evidence. Active secretion through the flagellar pocket of kinetoplastid flagellates is also well documented by other workers (Brooker and Vickerman, 1964; Seed *et al.*, 1967; Preston, 1969; Brooker, 1971).

The distended nature of amastigote-containing parasitophorous vacuoles provides

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Fig. 12. Electron micrograph of an infected macrophage labelled with colloidal thorium hydroxide. The parasitophorous vacuole (PV) is enlarged and contains label (arrowed). Labelled secondary lysosomes (LY) surround the parasitophorous vacuole, and some are fixed in the process of coalescence (also arrowed) (×9300).

Fig. 13. Electron micrograph of a macrophage infected with *L. m. mexicana* promastigote forms, and prepared for the histochemical localisation of acid phosphatase 48 hours later. Black deposits of lead phosphate (arrowed) indicate sites of activity. Parasitophorous vacuoles (PV) show only low activity, whilst an adjacent lysosome (LY) demonstrates a greater concentration of lead phosphate. The parasites (P) also contain vesicles which show acid phosphatase activity (×9000).

Fig. 14. Electron micrograph showing an uninfected macrophage prepared for acid phosphatase localisation. An autolysosomal vesicle (arrowed) shows a level of enzyme activity much greater than that found within parasitophorous vacuoles of similar size (×7500).

additional evidence for active interference with lysosomal enzyme activity. Steinman *et al.* (1976) describe how fluid influx into secondary lysosomes is encouraged if the lysosomes contain high molecular weight solutes that are impermeable to the vacuolar membrane. Normally such solutes would be degraded by lysosomal enzymes but if the enzymes are inhibited this could not occur, and the fluid within the secondary lysosome would be hyperosmotic. An ability incidentally to create an osmotic gradient into the parasitophorous vacuole could be advantageous to the parasite. It could assist the passage of nutrients into the vacuole, and help to incur eventual lysis of the macrophage, so releasing parasites for reinvasion of other cells.

The present investigation suggests that survival of *Leishmania* parasites in vertebrate macrophages is ensured by an active mechanism of protection that is 'switched-on' when transformation is completed. The mechanism probably involves blocking of the host lysosomal enzymes, within the parasitophorous vacuole, by substances secreted by the parasites.

SUMMARY

Infections of *Leishmania mexicana* in cultured normal mouse peritoneal macrophages show different morphological features depending on whether the parasites invade as promastigote or amastigote forms. Infections derived from promastigote invasion are characterized by parasitophorous vacuoles which develop slowly, and acquire only modest proportions. In contrast, the organisms in amastigote-derived infections lie within parasitophorous vacuoles which develop more rapidly, and attain a much greater size.

From observation of promastigotes of different species of *Leishmania*, it appeared that survival subsequent to endocytosis by normal macrophages depends on the parasites' rapid transformation to the amastigote form. Activation of the macrophage population produced an enhanced parasiticidal effect only against incompletely transformed *Leishmania* promastigotes.

Electron microscope investigations, involving enzyme histochemistry and lysosome labelling techniques, indicate that intracellular *Leishmania* avoid digestion by interfering with the activity of lysosomal enzymes that are freely delivered to the parasitophorous vacuole. It is proposed that this ability is acquired on transformation to the amastigote, and incidentally induces fluid distension of the parasitophorous vacuole through phenomena recently described by other workers.

ACKNOWLEDGEMENTS. We wish to thank our colleagues of the Department of Parasitology for valuable discussion on various aspects of this work and to the Department of Medical Illustration for assistance in preparing the plates.

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Enzyme activity within Leishmania parasitophorous vacuoles

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Recent studies (ALEXANDER, J. & VICKERMAN, K., 1975, *Journal of Protozoology*, **22**, 502–508; CHANG, K. P. & DWYER, D. M., 1976, *Science*, **193**, 678–681; LEWIS, D. H. & PETERS, W., *in press*) all indicate that host cell lysosomal enzymes are freely delivered into the phagosome (parasitophorous vacuole) containing the intracellular *Leishmania* parasite. The following study was therefore instituted to determine whether intracellular *Leishmania* avoided digestion by inactivating these enzymes.

Peritoneal macrophages from TFW outbred mice, infected with freshly isolated *L. mexicana mexicana* amastigote forms, were examined histochemically for localization of acid phosphatase activity. Preparations for the light microscope were incubated by the azo dye coupling method described by BARKA, T. & ANDERSON, P. J., 1962, *Journal of Histochemistry and Cytochemistry*, **10**, 741–762, and electron microscope preparations were incubated by a modification of the Gomori technique (LEWIS, D. H., 1976, *Doctoral Thesis, University of Liverpool*). Both methods revealed only a low level of acid phosphatase activity within the phago lysosomes containing *Leishmania* parasites, even though the parasites themselves showed strongly +ve localizations. Many non-infected secondary lysosomes were also weakly +ve, indicating that such may be a feature of "older" vesicles.

In addition, macrophages were allowed to phagocytose *L. m. mexicana* amastigotes in the presence of horseradish peroxidase (HRP). After a two-hour chase period, sites of HRP activity were localized by the technique described by STEINMAN, R. M., BRODIE, S. E. & COHN, Z. A., 1976 (*Journal of Cell Biology*, **68**, 665–687). Results showed that while non-infected cells contained numerous vesicles that were +ve for HRP activity, infected cells were generally only weakly +ve, and the parasitophorous vacuoles showed no activity. This may be attributable to a sequestration and neutralization of HRP from small cytoplasmic vesicles by the larger parasitophorous vacuoles.

These preliminary results, therefore, support the hypothesis of host lysosomal enzyme activity by *Leishmania*-derived factors. We believe such inactivation may indirectly cause the distension typical of *L. mexicana* parasitophorous vacuoles, through the osmotic phenomena described by STEINMAN *et al.* (1976).

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